

11 November 1982

Patching up the pipeline quarrel

The United States and Western European governments are trying to settle their quarrel about the Siberian pipeline. Their most important task is to find a way of rewriting the strategic embargo.

The great conflict between the United States and several industrial states in Western Europe about the pipeline intended to carry natural gas from the Soviet Union to the West seems to have entered a new phase — one in which statesmen wish they had behaved differently. Their immediate difficulty is that of knowing how best to retreat from positions they have established by earlier declarations that the pipeline dispute entails issues of principle that cannot be given up. The result is that a number of manufacturers that have supplied compressors for the Soviet pipeline are still on a kind of US blacklist, denied supplies of virtually anything from across the Atlantic. This circumstance is manifestly inequitable, if only because the dispute concerns not the companies themselves, which would have had to break binding contracts with the Soviet Union to fall in with the Reagan Administration's wishes, but their national governments. By now, there are obvious signs that the United States would willingly climb down from the hot-tempered position it assumed in the summer, but that it must have in return an agreement with Western Europe on trade with Eastern Europe and the Soviet Union. On what grounds can such an agreement be drawn?

As with other disputes, confrontation is more easily arranged than mollifying settlement. Both sides, the United States and West European governments, are affronted by the way in which their quarrel has blown up. In Europe, there is a widespread view that Mr Reagan should have said more plainly how strongly he and his colleagues felt about European participation in the pipeline project when they had a chance to speak their minds at the Versailles summit meeting last June. The United States, by contrast, claims to have been injured by the French government, which is accused of having misled it, but that complaint is insubstantial. The French government has made no bones about its determination to help with the pipeline ever since M. Mitterrand's election as President. It would have been especially hard to change course at about the time that the Soviet Union, under the terms of an agreement with France on scientific and technical collaboration, was planning to put the first Frenchman into an orbit about the Earth.

The difficulty in deciding where to go from here stems from the complexity of the underlying issues, and of the disagreements of principle that attend them. The most serious difficulty is that of dealing with the philosophical conundrum of knowing what the economic relationship should be between militarily hostile powers, the Soviet Union on the one hand, the United States and most of Western Europe on the other. In the old days, nobody bothered very much. France and Britain traded their way through the Napoleonic wars without much difficulty. The discovery that international hostility has economic dimensions dates only from the First World War, and from the recognition then that nation states could be brought to their knees by being physically deprived of goods, commodities and manufactures. Since the Second World War, this doctrine has been elaborated, perhaps further than logic will allow. At various times, both the United States and Western European governments have sought to distinguish between different kinds of goods: commodities embodying high-technology (Cray computers) have been on the West's strategic embargo list, but other materials (wheat, or less advanced computers) have been free.

One of the novel considerations that colour US policy on

relations with the Soviet Union is the recent recognition that the United States and Western Europe no longer have a monopoly of high technology (there is, after all, Japan), so that agreements that enable the Soviet Union to earn hard currency may make possible the purchase of high technology from outside the old magic circle. (This is the sense in which President Reagan is right to assert that his administration is not being inconsistent in agreeing that the farmers of the Middle West should continue to supply cereals to the Soviet Union, for that is a way of sucking up convertible currency, not for increasing the supply of it.) That calculation cannot, however, be central, even in Washington. If the pipeline does generate for the Soviet Union a steady cash flow in convertible currency, the result will most probably be an increase of general purchases from the West, wheat, meat, dietary fats at the top of the list but other simple manufactures as well. Especially because it is now plain that the Soviet Union will not have hard currency running out of its ears even when the pipeline is complete, and so will not be able to lavish support on its ambiguous (and dubious?) allies around the world, Washington's hard currency objection to the pipeline agreement is unconvincing.

Washington's argument can indeed be stood on its head. Detente in the sense of the early 1970s may be dead (with Afghanistan and, later, Poland), but the old struggle between two conflicting ideologies remains. So what better means of persuading Soviet governments and their dependants of the ideology that Adam Smith has been preaching for the past two centuries than to engage the Soviet Union in honest trade? Conversely, will not trade that is too tightly regulated put at risk whatever hope survives that the two systems may one day come to a durable understanding? In short, if the United States Administration is looking not for an agreement that it can present to Congress as a diplomatic triumph but for a basis for future understanding with Western Europe, it should stop complaining about the hard currency the Soviet Union will earn from building the pipeline.

The more substantial objection to European participation in that project is the complaint that Western Europe will become strategically dangerously dependent on the Soviet Union if it becomes too substantial a customer for Siberian gas. There is no denying that it is anomalous that governments planning how and where to give hospitality to a new generation of US missiles aimed at the Soviet Union should simultaneously be planning to make themselves to some degree dependent industrially on a natural product supplied from the Soviet Union. Quantitatively, the dependence of Western Europe on the pipeline will be marginal, but its symbolic significance cannot be ignored. Western Europe might therefore without dishonour agree with the Reagan Administration that participation in a second pipeline project could judiciously be postponed.

The much more difficult decision is how the West at large should regulate the transfer of high technology to the Soviet Union. The strategic embargo list is itself as anomalous as the central assumption on which it is repeatedly drawn up — that there is a hierarchy among different kinds of know-how, and that it is possible by means of, say, a strict embargo on trade in bicycles with gear-shifts to deny to some trading partner the opportunity ever to improve the performance of the direct-drive bicycles that he is allowed to buy in bulk. The same goes for mousetraps but



also computing machinery. The moral is that the Reagan Administration should now be bending its efforts to devising a system for a strategic embargo in which forbidden exports are classified not by type and catalogue number but by the effort in research and development that would be required to turn them into militarily useful weapons. On past experience, the United States would be chancing its arm by aiming at an advantage in time of three years or thereabouts. Does the present administration have the courage to acknowledge that that is about the best that can be hoped for?

Blow cold, blow hot

A relaxation of austerity has benefited the British scientific enterprise; but strings are attached.

The conclusion that the British Secretary of State for Education and Science, Sir Keith Joseph, may in reality be a better minister than he likes it to be thought emerged earlier this week from the Chancellor of the Exchequer's forward look at public spending in the three years immediately ahead. Sir Keith seems to have won from the Treasury an extra £2 million, or 0.4 per cent, of the budgets of the research councils in the financial year immediately ahead (beginning on 1 April next year). But since, unexpectedly, it has become known that an extra £4 million will be spent next year on the British Antarctic Survey, there is no way of telling whether the Natural Environment Research Council (the survey's sponsor) will be deprived of a further £2 million. Plainly, all departments of state in the British government are still fighting the Falklands war (which is not to say that the Antarctic survey will not put the extra funds fallen into its lap to good use).

The more valuable component of the science budget for next year, as now amended by the British Treasury, is what was being said earlier this week about the special support to enable universities to recruit young people to academic posts. Sir Keith has already stolen £6 million from the budget of the Social Science Research Council for this purpose, but when that will become available is to some extent in the hands of the research council itself. Now the intention is that up to £10 million should be spent on the recruitment of talented people (mostly in the sciences) to posts in the universities. For the time being, the fact that it is by no means clear how the extra money will be spent will not shade the joy with which university campuses will be suffused. For the best part of a decade, British universities have been unable to recruit to their teaching staffs the talented people whom, in other times, they would have snapped up. Even the most secure among older academics now acknowledge their need of "young blood". So, it should be said, they should have done some time ago.

Apart from the uncertainty about the source of this extra money, and its duration, the only difficulty in what the Department of Education and Science was saying this week after the Chancellor's declaration was that some of the extra money to be channelled towards the universities will be spent on courses in information technology. Apparently Sir Keith Joseph has been listening to Mr Kenneth Baker, the minister of state at the Department of Industry with special responsibility in this field. While Mr Baker is dangerously oversold on this government's plan to install cable television in Britain any day now (see *Nature* 28 October, p.765), he is right to be plugging the virtues of the technology of information. The open question, which Sir Keith Joseph should be brooding about, is whether it is right (let alone proper) for the Department of Education and Science to be telling universities that they can have extra money provided that they toe some ministerially determined line. In their hearts they know they should resist, and collectively refuse the money. In their boots, they know there are enough among them who will accept to undermine whatever high-minded position the high-minded among them might adopt. In short, the universities are being told that there will be graduate courses on information technology even if individual universities see no need of them. Sir Keith's success in persuading the Chancellor of the Exchequer may, in other words, have designed a poisoned chalice for them all.

Do not foretell the future

The European Commission's futures research project has not seen the future; but nobody can.

In Brussels, FAST means "forecasting and assessment in the field of science and technology", and the European Community now has two substantial volumes of a report on the subject. Among the now copious literature of the future, the report stands out for its sanity — the guiding assumption is that there must be a way in which present economic troubles can be cured by intelligent research, development and capital investment. The document is also full of interest — there is an intelligent discussion of the changing pattern of employment under the influence of changing technology and of what the authors of the report repeatedly call "the international division of labour", usually in a European context. Moreover, recommendations as to where, in the whole field, research and development should be encouraged are not pulled like rabbits out of a hat but are, rather, preceded by a careful account of the present problems of European industries, from chemicals to construction. For all these virtues, however, the report is too disjointed to be persuasive. Worse still, it is unlikely to be the kind of touchstone for planning the future development of the European Community's research programme that the Commission hoped for when the work was set in hand three years ago.

The reasons for this disappointment are easily found, and are probably inescapable in any attempt such as this to foretell the future. First, the forecast provided, necessarily a projection of the present into future decades, is innocent of the surprises yet to come. In this spirit, the FAST report pays particular attention to two now fashionable fields in which technology is likely to have a profound effect on the character of society — information technology and biotechnology. Thus the report has much in common with other recent attempts to plan ahead in research and development, the attempt by a committee of the British Department of Industry to map out a programme of research and development in information technology, for example. Thus, while some seers have for years been prophesying that biotechnology will be important, it is less than ten years since the prospect became as substantial as it is now. Nobody can be blamed, but even the specific account of the future now offered must overlook the technologies that have not yet emerged. A further snag is that committees are now well placed to plan the future strategy of research. Yet while committees have been saying for the past thirty years that the closing decades of this century would be dominated by the technology of telecommunications and computers, it has never previously been possible to spell out just how a government or a corporation should seek to profit from the likely course of development.

For such reasons, it is to be hoped that the European Community as a whole will take most to heart those features of the report now published that deal with structural questions, not the details of which research and development projects are likely to be most valuable in the years ahead. First, there is an urgent need for more incentives, within the Community, for a rational division of labour that will allow the most efficient producers to produce most. Although there has recently been some progress in throwing public purchasing open to all European companies, governments are still able to drag their feet in the interests of chauvinism — and as a result undermine their own long-term prosperity. Similarly, there is an urgent need somehow to make the European system of higher education and technical training more flexible, and to encourage mobility within it. Here again, chauvinism, if not outright xenophobia, is still all too common within Europe. But would it not make great sense that Europe should, for example, work towards some common university system? On both counts, alas, the obvious objection is that if some nations gained from a more efficient division of labour, others would lose. The answer is that this is not a "zero-sum game" but one in which the wealth of Europe as a whole would be improved.

Further snag with Stanford patent

Filing error claimed in Cohen-Boyer

Washington

The second Cohen-Boyer patent covering basic processes in genetic engineering may have suffered another setback last week when attorneys for the applicants admitted that there is a serious error in the patent specifications.

The error was one of several potential flaws cited by the US Patent and Trademark Office when, in August, it suddenly reversed its previous decision and decided to reject the patent (see *Nature* 12 August, p.595). Responding just one day before a deadline of 2 November, Stanford University and the University of California concede the error, but argue that the patent is nonetheless valid.

Their response, however, may raise more questions than it answers. Attorney Bertram Rowland, representing the universities, does not explain why he failed to inform the patent office of the error, which, he admits, "was known to the applicants' attorney as early as December 1977". And the two lines of defence he introduces in support of the patent's validity may well be disputed.

Rowland argues that despite the error, the patent office should withdraw its

objections and grant the patent, and he offers two lines of defence for that argument. The first is that even "if applicants had not actually performed any of the experiments described in the experimental section . . . the specification would be enabling (sufficient to allow duplication and thus be patentable), since it teaches how to obtain and clone a functional plasmid containing foreign DNA". In other words, pSC101 is not itself vital, as the instructions provided in the patent explain how to select, isolate and use other suitable plasmids. "Testimonials" from molecular biologists Donald Helinski, Stanley Falkow and Bernard Weissblum are included to buttress the claim that anyone with "ordinary skill" in the field could, since 4 November 1974, have used the patent to prepare a functional plasmid — even without the benefit of a correct recipe for pSC101.

Yet in a submission to the patent office on 24 June 1977, Rowland seemed to take the opposite view — that pSC101 was indeed vital to the patent claims, and that this "new plasmid found by the inventors" was what distinguished their claims from potentially competing work by others.

The second line of defence offered by Rowland is that even if pSC101 is the key, the patent does not need to disclose how to make it since pSC101 was publicly

available at the filing date. A declaration by Cohen, included in the response, states that he made the plasmid available to scientists, subject to two restrictions: "that it would not be passed on; that it should not be used in experiments believed at the time to have the potential to be dangerous". Cohen asserts that "no-one who requested pSC101 was denied pSC101" and that it was thus "widely available".

One patent attorney familiar with the case said that Cohen's restrictions on distribution of the plasmid may defeat this defence; to be safe, he should have deposited the plasmid in a recognized public repository such as the American Type Culture Collection before filing. This was not done until 25 June 1981, six months after the first patent was granted.

Rowland's admission of the error in the second patent application raises a potentially explosive question: should the first patent, which contains the same flaw, be reissued to correct it? Given the problems with the second patent, this is something Stanford would like to avoid. In an interview last week, Rowland tried to avoid the issue, saying, "Given that 99 per cent or more of the people in the field are aware of the situation [the error in the pSC101 recipe], whether I should have to go to the expense of filing for reissuance is an open question". He added, "I don't

UK research councils' budget break

British research councils were told this week how much money they will get next year, but not which council is getting what — surely a formula for a scrap until the detailed allocations are announced in a few weeks' time.

According to Sir Keith Joseph, UK Secretary of State for Education and Science, who made a statement to the House of Commons on Monday, the total to be made available is £509.7 million, a shade above the £507 million requested by the Advisory Board for the Research Councils (ABRC) a few weeks ago (see *Nature* 4 November, p.7). However, the new figure includes a Falklands factor — an extra £4 million for the British Antarctic Survey (BAS), in line with policy announced after the Falklands conflict to increase research in the Antarctic. BAS is a component institute of the Natural Environment Research Council (NERC). NERC would welcome the sum as a gift, a spokesman said this week, but would be worried if it were to be subtracted from its planned budget for other science, or from that of other research councils.

Another unresolved issue is the position of the Agricultural Research Council, which has protested against ABRC's recommendation to hold its budget constant in cash terms for the next three

years at £46 million.

As for the Social Science Research Council, "a small portion" of the £6 million cut threatened recently by Sir Keith Joseph will fall this year, with more to come in the next two years. It will contribute to a £14 million fund to provide "the first instalment of a programme for new technologies, including the recruitment of young researchers". Of this, £4 million will go to establish courses in information technology in technical colleges and polytechnics. The remaining £10 million is for new blood as well as for the provision of courses in new technologies "primarily to improve the supply of manpower in information technology". Moreover, part of the sum will be allocated not by the University Grants Committee and the universities, but by the research councils, said Sir Keith. So it is impossible to determine exactly how many jobs this £10 million will make available. A separate announcement will give details.

As for the universities themselves, they will enjoy a small increase in their grant for the present academic year (1982-83) from £1,137 million to £1,150 million, and the recurrent grant for 1983-84 will be set at £1,213 million. These figures are fixed in cash terms, and assume a salary increase of only 3.5 per cent.

Robert Walgate

Rowland's concession concerns an article published in November 1977 by co-inventor Stanley Cohen and a co-worker (Cohen, S.N. & Chang, A.C.Y. *J. Bact.* 132, 734; 1977) which contradicts the recipe set out in the patent for making pSC101, the key plasmid used for inserting foreign DNA into *Escherichia coli*. "pSC101 could not be prepared in accordance with the working example", Rowland writes.

The *Journal of Bacteriology* article came to the attention of the patent office only this summer, apparently through an article by an Exxon patent attorney, Albert Halluin. In several footnotes to a chapter he contributed to *The Patenting of Life Forms*, a book published in August by Cold Spring Harbor Laboratory, Halluin cites that article and other potential flaws in the patent. Rowland, after admitting that he knew of the existence of Cohen's article five years ago, writes that "I have no recollection as to why it was not brought to the Examiner's attention in the written record". The patent application was filed on 4 November 1974; it was later split into two parts, and the first patent was granted on 2 December 1980.

know any law that says I have to'.

One old question that has resurfaced in the latest exchange between Stanford and the patent office, and that may bear on the first patent, is whether Cohen and Boyer are the sole inventors, as Stanford and the University of California claim. In its notice of rejection on 2 August, the patent office cites a statement by Dr Robert Helling — a co-author of the 1973 paper in the *Proceedings of the National Academy of Sciences* that is the basis of the patents that appeared in a news story in the 3 April 1980 issue of *Nature* (p.388). Helling, now at the University of Michigan, is quoted as refusing to sign a disclaimer of inventorship as requested by Rowland.

Helling has not, however, pressed any claims to date. And Rowland, in his response to the patent office, asserts that Cohen and Boyer are the sole inventors, having conceived the idea at "a now famous delicatessen in Hawaii" during a scientific meeting in November 1972. This, Rowland writes, rebuts "any implication that the refusal to sign a disclaimer might be equated with an allegation of co-inventorship".

But Helling told me last week "I certainly am a co-inventor. The three of us were equal partners. I went out there to

develop new cloning procedures; Herb (Boyer) was the one who suggested using plasmids, and asked me if Stan (Cohen) could join us". Helling worked with Boyer at the University of California at San Francisco during his 1972–73 sabbatical.

Helling has been discussing the matter with the University of Michigan's attorney and said "we may do something now", but declined to give any details. A call to the attorney was not returned.

Although a recent court decision overturns the patent office's policy of considering co-authorship *prima facie* evidence of co-inventorship, Helling's statement in the 1980 *Nature* story complicates the matter.

Rene Tegtmeyer, the assistant commissioner for patents, said his office will act "promptly" on the case, perhaps reaching a decision within "two or four weeks". If the patent office stands by its rejection on 2 August, Stanford will then have three months to file a request for reconsideration or a notice of appeal to the Patent Office Board of Appeals. A decision might not be reached before the summer of 1984 — and the case could go on still longer if the board's decision were to be referred to the Court of Appeals for the Federal Circuit.

Stephen Budiansky

research grant applications are being maintained".

Professor I. Butterworth, chairman of SERC's Nuclear Physics Board, is more pointed, saying that the board wants SERC to provide a certain number of permanent posts in nuclear physics attached to universities until UGC is once again able to play its part.

Since 1978, the proportion of support for nuclear physics within SERC's overall budget has dropped from more than 30 per cent to about 20 per cent. The intended opening at Daresbury of the Nuclear Structure Facility (a heavy-ion accelerator) after an extended delay will provide some stimulus but, according to Professor Butterworth, its full utilization will be delayed. There is also a fear that if full support is given to LEP, the electron-positron collider at CERN in Geneva, it may not be possible to fund other particle physics experiments.

To judge from the report, physicists are also concerned that support for such fields as atomic spectroscopy, semiconductor research, superfluidity and superconductivity appeared to be decreasing relative to such fields as biology, chemistry and applied mathematics. Apparently a special committee has been considering criteria to be used in deciding the relative balance of support between different fields, but its conclusions have not been made public. And there is a possibility that the Synchrotron Radiation Source at Daresbury, from which X-ray spectroscopists have derived particular benefit, may be affected by rising costs, which may make necessary a choice by the Science Board between research grants on the one hand and further development and general support of central facilities on the other.

Support for information technology and space science, both recommended by the Advisory Board for the Research Councils, are, however, both booming at SERC. The two fields are beginning to link up, with the recent development of remote control of telescopes via satellite links between the Royal Greenwich Observatory and telescopes in Hawaii. Such developments might pay for themselves in reduced travel budgets for astronomers.

On the more contentious issue of the use of the South African Observatory (see *Nature* 23 September, p.291), the council says it is not in the business of taking political decisions at the expense of the interests of science and that, moreover, it had felt little pressure from the community and none from the government to sever its links with its South African equivalent.

The press conference called by SERC was made all the more bland by the decision not to make the report available in advance. Thus SERC, proud of its autonomy, has followed a government directive resulting from the premature publication in the national press of details of medals awarded for the Falklands fighting.

Philip Campbell

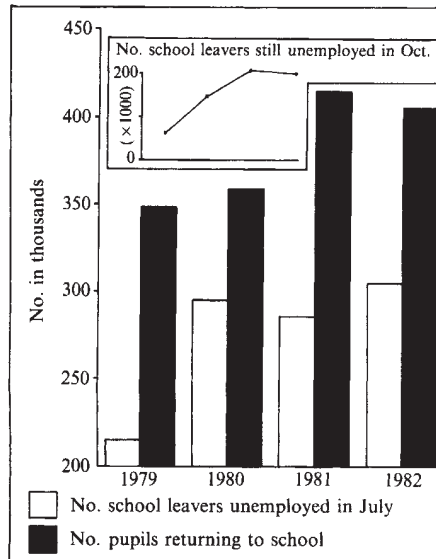
Science and Engineering Research Council

Big money for big science?

A potentially important difference of emphasis between the British Science and Engineering Research Council (SERC) and the Advisory Board for the Research Councils (ABRC) was apparent at the publication of the research council's report (HMSO, £4) last week. For while the advisory board said in its advice to the Secretary of State for Education and Science that the time had come to halt the retreat from Big Science (see *Nature* 4 November, p.1) SERC chairman Professor John Kingsman refers to "broadening out from the support of 'big science' to a better

spread of science and engineering". Kingsman says that the advisory board's recommendation would be only one element in the council's decision how best to spend its money next year.

SERC's principal area of concern is the dual-support system through which funds for science research are provided jointly through the University Grants Committee (UGC) and the research councils. The SERC report says little about how far SERC is prepared to go to compensate for the dwindling UGC contribution, but also claims that "the quantity and quality of



As the probability of long-term unemployment for the British school leaver has increased (reflected in the October unemployment figures), the number of pupils returning for further education has gone up. There has been no increase in resources made available to schools and extra pupils are being swallowed up by existing classes. However, fears that this would lower the standard at GCE 'O' and 'A' level are not borne out by statistics published by the examining boards, which show no real change in the pass rate during this period. The decline in numbers returning to school in 1982 is at least partly due to the rapid expansion of the Youth Opportunities Programme during 1981–82 from 360,000 to 553,000.

Melanie Kee

US elections

Key posts held

Washington

Last week's congressional elections, which gave the Democrats a 26-seat gain in the House of Representatives, returned all but two of the senators and congressmen who hold key positions on science and environment committees. The two losers were Senator Howard Cannon (Democrat, Nevada), the ranking Democrat on the Commerce, Science and Transportation Committee, and Senator Harrison Schmitt (Republican, New Mexico), a former astronaut and the only PhD scientist running for the Senate.

Cannon, who lost in a close race in his increasingly Republican state, has been a senator for 24 years. He was recently instrumental in pushing through legislation to guarantee continued funding of the Landsat remote-sensing programme.

Schmitt, who is completing his first term, has been a staunch supporter of President Reagan's economic policies, although he recently went against the Administration in sponsoring legislation to increase spending on science education. Schmitt also supported a shift in funding from the Department of Defense to the National Aeronautics and Space Administration for shuttle operations and for increased space and aeronautics research.

Three supporters of environmental legislation in the Senate who faced strong opposition in the election will all be back: Robert Stafford (Republican, Vermont), George Mitchell (Democrat, Maine) and John Chafee (Republican, Rhode Island).

In the House of Representatives, all the key players on science and the environment committees will be returning, although George Brown (Democrat, California), a strong advocate of science education and research, was pushed to sending out a plea during the campaign for financial support from scientists and educators.

At the other extreme, Senator William Proxmire (Democrat, Wisconsin) of Golden Fleece Award fame proved much more popular among his constituents than among the nation's scientists. He wound up with 64 per cent of the vote, having spent less than \$150 on his re-election campaign.

A resolution calling for immediate negotiations toward a bilateral nuclear freeze was passed in eight states. Although the resolutions are not binding, the Reagan Administration had campaigned heavily for their defeat, arguing that a freeze would endanger US security. Voters in Massachusetts, Michigan, Montana, New Jersey, North Dakota, Oregon and Rhode Island passed the measure by a wide margin. It narrowly passed in California and failed in Arizona, the only other state where it was on the ballot paper.

Stephen Budiansky

West Germany science

Federal plans

The implications for science of the change in government in West Germany are emerging only gradually. The new CDU/CSU/FDP coalition is clearly going to be cautious in what it does at least until after the federal election next March. In any case, the vast and decentralized structure of German science makes it impossible to implement changes quickly. An indication of the new team's plans has been given by Dr Heinz Riesenhuber, CDU minister at the Research and Technology Ministry (BMFT) in speeches at a joint industry/BMFT seminar on technology transfer and at a meeting of the Fraunhofer-Gesellschaft in Munich.

Dr Riesenhuber made it clear that direct support of individual projects will be run down in favour of indirect support through tax relief, low interest loans, allowances and subsidies. The rundown can be seen as a continuation of the previous administration's policy under which barely half as many projects were started in 1981 as in 1979. Dr Riesenhuber hopes that indirect support will encourage more market-oriented research and he singled out the energy saving programme for particular criticism, saying that the returns for the DM 4,350 million (US \$1,700 million) spent are quite inadequate.

Interferon therapy

Side effect scare hits French trials

Paris

Four patients out of eleven being treated for cancer with French alpha-interferon have died from the same cause this year in France — myocardial infarction, a failure of a section of heart muscle through the loss of local blood supply. As a result, Jack Ralite, the French minister of health, has cancelled the trials, at least until Institut Pasteur Production (IPP), which produces the drug, can repurify its stocks and perform new toxicity tests on the material.

The deaths could be attributable to the interferon itself, or to impurities in it, or it could be just a run of bad luck. Several other countries are performing human trials with interferon and there have been no reports of the heart disease syndrome, and IPP uses essentially the same production method as Dr Kari Cantell, who has supplied much of the world's alpha-interferon: extraction from blood-bank leukocytes.

But the sequence of four similar deaths was too much to ignore. The scientific council which was set up to advise Ralite specifically on trials with the IPP interferon (the only French source) advised him to halt the trials. According to the president of the council, medical statistician Professor Robert Flamant,

In a similar vein, at the technology transfer meeting, the minister demanded that work at the 12 large state-supported research centres should concentrate more on market-oriented work and suggested that there should be more independent co-operation between research and industry. He expressed a belief that there is a pool of research results which could lead to industrial innovation at little or no cost. This seems questionable, for while it may have been possible to carry out development on a shoestring fifty years ago, modern requirements for quality and reliability in products and processes have made the step from research to production both expensive and time-consuming. If Riesenhuber's pronouncements are a foretaste of the kind of shift in emphasis that has occurred in science budgets in the United Kingdom and in the United States during the past decade, scientists working in basic research and on long-term projects will have to fight hard for their corners.

In more concrete terms, the draft budget for 1983 shows that the immediate effects will be small. The total research budget is DM 6,911.8 million (US \$2,710 million), a decrease of 2.4 per cent compared with the draft budget of the previous government and of about 2.2 per cent compared with 1982. Details have not yet been announced and the proposals may be modified when they are discussed in the Bundestag on 11 November.

J.S. Dunnett

suspicious were first raised when the first two patients both died of myocardial infarction near the end of their treatment. They were both women 50–60 years old with acute breast cancer, who had been treated by other drugs and with radiation. The deaths led to "discussions" in the council, but they were dismissed as coincidence.

There was no problem with the third patient treated — nor any cure. But the fourth, a kidney cancer case, died of myocardial infarction actually during one of the interferon infusions.

At this point the council decided to change the method of introduction of the drug, which until then had been the "more aggressive" intravenous route rather than the intramuscular route which is usually preferred but which limits the size of the doses. This was fine for six patients, but in the seventh, with a slight increase of dose, there was another myocardial infarction. It was "less clear" in this case that interferon might be to blame, as the patient had a history of slight heart trouble. But it was enough to lead the council to stop the tests, especially since they also noted that a child had suffered great shock — and just survived — when given an injection of the interferon for a virus infection.

Flamant, however, expects his council to reauthorize the trials when IPP has

repurified its stock (leukocyte interferon is always contaminated with various other proteins besides interferon) and if the toxicity trials — to be performed on dogs — are completed successfully. This may take two months, he estimates.

There is anxiety, however, about non-French interferon, over which Flamant's council and the minister himself have little or no control. The council deals specifically and solely with IPP interferon because it arose as a *quid pro quo* when the government first offered a guaranteed market to IPP for its product. Having guaranteed the cash, the government also wanted to ensure proper statistical control of the trials and so established the council.

Anglo-Australian bomb tests

Government traces victims

Canberra

It seems that the dust has not settled over Maralinga or at a site 200 kilometres to the north of it called Emu, both in South Australia, nor over the Monte Bello Islands off the coast of Western Australia. It was in these remote spots that the British government conducted a series of 12 atom bomb tests between 1952 and 1957 (see table). In addition, in the following six years, weapon experiments involving radioactive fallout, though not from explosions, were undertaken at the testing range of Maralinga — an aboriginal word meaning "field of thunder".

The federal government is now trying to trace people associated with the tests, some 15,000 in all, in order to establish if there might be a link between mortality and morbidity of this group and the tests. On 22 October, the Minister for National Development and Energy, Senator Sir John Carrick, announced that questionnaires seeking medical data were sent to all the 8,000 people whose whereabouts are known through sifting administrative records, and he appealed for help to find the rest. The Department of National Development and Energy, which is conducting the survey, is also unearthing death certificates for information on the number and causes of death among this population. Furthermore, the government

But non-French interferon enters the country with no more than an import licence — and some of it, it is suspected, without any licence at all. This foreign interferon is being used for phase 1 and phase 2 human trials without any control, and with little knowledge on the part of the ministry.

It is therefore being suggested that the foreign interferon material, and other experimental drugs as well, should be controlled under a comprehensive oversight scheme. Thus questions raised over the purity of a particular kind of French interferon may also affect other drugs, both French and foreign.

Robert Walgate

commissioned an independent body of experts, the Australian Ionising Radiation Advisory Council (AIRAC), to examine the adequacy of safety precautions at the time and the possibility of ill effects from fallout. This report, now in its final draft, was expected to be released in a month but it will now be delayed until next year, partly because it contains some classified information awaiting clearance by British authorities.

The survey is both a response to and an attempt to allay growing public disquiet in the past three years over newspaper reports, mainly from South Australia, of illness and death among servicemen involved in the tests. Another story, just as alarming, which may not be apocryphal, is about a tribe of the Pitjantjatara aboriginals wandering into ground zeros during the tests unbeknown to the British. An attempt by the South Australian Health Commission to authenticate that claim only served to highlight the difficulties of obtaining information from a small nomadic and dispersed group and the lack of control data for comparison.

Of the many compensation claims lodged at the office of the commissioner for employee compensation, liability has been acknowledged in 5 cases — 3 for deaths from cancer, 1 for the aggravation of an existing nervous complaint, and 1 for

a thyroid disease. The largest sum, A\$32,000, was paid to the widow of Mr Frank Eaglen who died of cancer. Claimants are aided by the fact that the onus of proof rests with the government.

Australia and the United Kingdom agreed to establish a testing range at Maralinga in August 1954 and it was approved by cabinet in May 1955. Subsequently the Atomic Weapons Safety Committee was instituted, whose job it was to ensure that the tests were conducted to the satisfaction of the Australian government. This committee of scientists had the power to veto, until the moment of firing, any test not meeting its safety criteria. After its closure, the range was partially decontaminated in 1964 and again in 1967 (operation "Brumby") by British teams. However, as a consequence of Australia's ratification of the nuclear non-proliferation treaty, Britain was asked to "repatriate" half a kilogramme of plutonium existing as a single discrete mass buried at Maralinga and the material was removed in 1979, the same year in which the Department of National Development and Energy completed a programme of waste management and rehabilitation in accordance with AIRAC recommendations.

Vimala Sarma

Radiation exposure

Unions agree

A scheme for compensating the dependants of radiation workers who have died from cancer has been agreed between British Nuclear Fuels Ltd (BNFL) and its trade and staff unions. The scheme will apply to present and past employees, but requires that dependants will be eligible for compensation only if there is evidence that the cancer may have been caused by occupational radiation exposure.

British Nuclear Fuels is the publicly owned monopoly for reprocessing and fabricating nuclear fuel. The distinctive feature of the new agreement is that compensation will be determined by the probability that a cancer has been induced by radiation, thus avoiding the "all or nothing" conundrum that has complicated earlier legal cases.

The scheme is hailed as a "pioneering deal" by Mr John Edmonds, National Energy Officer of the General and Municipal Workers Union (the main union involved in the negotiations). It is certainly a unique scheme in the nuclear industry, although large employers in other industries run compensation schemes that work along similar lines. Previous claims in respect of radiation-induced disease made through the courts have resulted in very long delays in payments; several substantial out of court settlements have been made.

The new procedure is voluntary, but the unions involved will recommend claimants to make use of the scheme. The option of taking a case to court instead is not

UK atomic tests in Australia 1952-57

Code	Location	Firing site	Date	Size
Hurricane	Monte Bello	Off Trimouille Island	3 Oct 1952	Kilotonne
Totem 1	Emu	—	15 Oct 1953	Kilotonne
Totem 11	Emu	—	27 Oct 1953	Kilotonne
Mosaic G1	Monte Bello	Trimouille Island	16 May 1956	Kilotonne
Mosaic G2	Monte Bello	Alpha Island	19 June 1956	Kilotonne
Buffalo	Maralinga	One Tree	27 Sept 1956	Kilotonne
Buffalo	Maralinga	Marcoo	4 Oct 1956	Low yield
Buffalo	Maralinga	Kite	11 Oct 1956	Low yield
Buffalo	Maralinga	Breakaway	22 Oct 1956	Kilotonne
Antler	Maralinga	Tadje	14 Sept 1957	Low yield
Antler	Maralinga	Biak	25 Sept 1957	Kilotonne
Antler	Maralinga	Taranaki	9 Oct 1957	Kilotonne

Source: Australian Ionising Radiation Advisory Council Reports Nos 4, 5 and 7 tabled in the House of Representatives on 30 May 1979, 13 November 1979 and 22 May 1980 respectively.

excluded, and a compensation payment under the new procedure does not constitute an admission of liability by BNFL.

BNFL is to examine radiation dosage records of its employees at Sellafield (formerly Windscale) and its other plants, and will include employees of its predecessors in the field, the Atomic Energy Authority and the Ministry of Supply, going back to 1950. A BNFL spokesman, while emphasizing the company's continuing efforts to minimize radiation exposure among its employees, estimated that between 10 and 40 additional pay-

ments are expected immediately under the new scheme.

In difficult cases an independent panel of experts, jointly appointed by BNFL and the unions and under the chairmanship of Lord Gregson, will decide the level of compensation. The agreement is provisionally scheduled to last for two years and will be extended if it proves successful. It is also hoped that it might be possible to extend the scheme to cover cases in which radioactive material has been ingested (more difficult to assess for technical reasons) and cases of disablement. **Tim Beardsley**

Thermonuclear fusion

Padua approved

Brussels

It is now virtually certain that the reversed field experiment (RFX) which was originally to be funded by the UK Atomic Energy Authority at the fusion research centre at Culham, will be taken on by the Italian Comitato Nazionale Delle Ricerche and built at its laboratories in Padua. The consultative committee of the fusion programme was given the go-ahead to both this project and the construction of the Wendelstein VII advanced stellarator project, to be built at the Garching Laboratory of the Institut für Plasmaphysik in Germany.

Researchers were disappointed last year when the British government decided that there was no room in Culham's budget for the British contribution to the experiment of 16.5 million European currency units (ECU, 1 ECU = 55p). RFX is a direct descendant of ZETA — the zero energy thermonuclear apparatus — which hit the headlines in the late 1950s when it failed to achieve fusion for reasons which were not understood for some years. G.B. Taylor in the next decade proposed the reverse field theory to explain the failure and it was hoped that Britain could continue to lead the world in this field.

Reversed field pinch is one of the three options for achieving fusion which are being pursued in Europe under the Community's research programme. The experiment which will now be started in Padua will involve the construction of a 2,000 kA machine which will enable the experimenters to achieve a longer plasma confinement time. Padua had in 1978 been successful with a much smaller machine, known as ETA-BETA, capable of producing plasma currents up to 250 kA.

The project will still involve staff from Culham but the Italians will be providing the bulk of the cost of the experiment, some 15.7 million ECU, which will start next year and cover about four and a half years. The Community's budget will meet the rest of the cost, totalling 28.5 million ECU although this may rise, depending on whether the US Department of Energy is still willing to contribute to the cost of the project.

Officials in Brussels are, however, confident that the project may now go ahead without further setbacks. In fact, there may be advantages in siting the experiment in Padua where it will not have to play second string to the JET tokamak.

The Wendelstein VII advanced stellarator project will be the first stellarator to use modular twisted coils instead of helical windings to create the poloidal half of the magnetic field that heats and compresses the plasma. The stellarator will cost 12 million ECU to build and the Community will be contributing 5.4 million ECU.

Jasper Becker

EEC research council

British block safety reactor

Brussels

An otherwise successful meeting of the EEC's research ministers in Brussels last week was marred by some tough British objections to the new budget put forward by the Commission for the Super-Sara project. The project, which aims to explore ways of managing crises in light water reactors when loss of coolant takes place, is included in the new four-year (1982–86) programme for the EEC's joint research centres (JRC). Super-Sara will absorb a considerable part of JRC's budget at a time when efforts are being made to restructure its work so that it is more in line with other research and development aims of the Community.

Super-Sara was first proposed after the Three Mile Island accident and when the Italians wished to find a use for the Essor experimental reactor which was no longer being used at the Ispra Research Centre. Since then the enthusiasm of the rest of the member states, never very strong, has waned considerably as the original cost estimates have been continually revised upwards. British officials were predicting before Thursday's council that the project could be scrapped and even now its future is by no means certain.

A decision will have to be taken by Coreper, the committee of the member states' ambassadors, within the next two weeks before the revision of the 1982 budget is completed. Mr David Mellors, British Under-Secretary of State for the Department of Energy, has now asked the European Commission to present further details on how the increased project costs can be accommodated and has questioned whether the project, which he thought had been badly managed until now, could still be considered cost-effective.

Mellors said that the results that the project would yield could not be considered essential since it investigated only an extremely unlikely type of malfunction. The Commission has, however, received support from a task force of experts whose report concluded that the project was worthwhile and fitted into the matrix of research being carried out elsewhere. And even the United States has until now shown

interest in contributing to the cost of the project. British opposition might be rooted in national interests because, having chosen to build pressurized water reactors, the United Kingdom will be only marginally interested in the results of the project.

Considerable confusion however, surrounds the various estimates of Super-Sara's cost. An early estimate of 139.53 million European currency units to cover the project until 1988 has been constantly revised, standing now at 293.9 million units (£161.7 million).

European Commissioner Viscount Etienne Davignon told the ministers that 10 million ECU and 17.5 million ECU urgently needed to be taken from the 1982 revised and 1983 budgets respectively, plus a further 15 million ECU each for 1984, 1985 and 1986, if the project was to continue at all. He also pointed out that more delays will increase the cost of the project still further and that the cost of closing down the Essor reactor would not be cheap either.

Part of the explanation for the dramatic rise in cost estimates lies with the nature of the revision of JRC's programmes. Next year will be a special bridging year between the last programme (1980–83) and the 1984–87 programme. More staff are being recruited and 161 new posts are being created, 81 of them for the Super-Sara project, although the Commission claims that this is a temporary expansion to cope with the expected retirement of senior scientists. It now seems likely that instead of new staff being engaged there will have to be redeployment of staff among JRCs and national laboratories or certain other projects or plans will have to be curtailed such as the increase in JRC research asked for in nuclear energy. Except for the possibility of the number of experiments being reduced, there appears to be very little room for compromise over Super-Sara itself, and the British are worried about putting too many eggs in one basket. Even under present calculations, the fast breeder safety and the fuel cycle safety programmes are being cut back.

Jasper Becker

NSF gets new director

Almost as soon as John B. Slaughter, the outgoing director of the US National Science Foundation (NSF), the nation's basic research agency, announced that he would leave on 1 November to take up his duties at the University of Maryland, the White House announced his successor. On 3 November Edward A. Knapp assumed his new duties as director.

Knapp, a physicist with degrees from Pomona College and the University of California at Berkeley, had been confirmed in September as assistant director of NSF for mathematical and physical sciences. His entire previous career was with the Los Alamos National Laboratory, where he worked with George A. Keyworth, who is now the President's Science Advisor. Ironically, the White House personnel office made the appointment with despatch while Keyworth, Slaughter and NSF's deputy director Donald Langenberg — who was rumoured as a likely candidate — were out of town.



Knapp's work at Los Alamos began in 1958. He worked on controlled thermonuclear research and helped to plan the linear proton accelerator, the Los Alamos Meson Physics Facility (LAMPF). Knapp spent a year at CERN in Geneva, where he worked out a collaborative programme between CERN and Los Alamos. Before coming to Washington Knapp was director of the laboratory's accelerator technology division.

Since Congress is in recess at present, Knapp is assuming his duties without being formally confirmed. Langenberg plans to stay on, although his name is on a list of candidates for the presidency of the University of New Mexico. At the time that Knapp was appointed, there were rumours in Washington of a major conservative housecleaning at NSF, which could not be confirmed. Moreover, the national elections, which seemed to deprive the Reagan Administration of some of its far-right conservative mandate, make such a sweep very unlikely. **Deborah Shapley**

French energy

Forecasts fall

In the midst of an appalling economic crisis, the French government is about to be struck another blow with the expected publication this week of an official energy forecast which will lower projected electricity consumption in 1990 by nearly a quarter.

The consequences fall neatly on the coal and nuclear industries, both of them political hot potatoes for a socialist government. Coal and nuclear energy are the dominant power sources in France (producing 24 per cent and 37 per cent of delivered electric power in 1981), excepting hydroelectricity (27 per cent) which, as nearly "free" power, is likely to be untouched by the new forecast. The socialist party has committed itself to support the coal industry — and indeed expand production — but the nuclear lobby is strong and supported vociferously by the powerful research and industry minister, Jean-Pierre Chevènement.

The new figures come from the Commission du Plan, an institution which is both within and to some degree aside from the government. But its pronouncements — broad political and economic plans for the future — are taken seriously. In this case, the "long-term energy" committee of the commission has calculated that electricity consumption will not be 450,000 million kWh in 1990 as envisaged in the previous (1980) plan, but only 350,000 million kWh, compared with a true consumption in 1980 of 246,000 million kWh. Thus the projected increase has been halved, with a consequent revolution in thinking about power station construction and power sales.

Electricité de France (EDF), which is strongly behind nuclear power, contests the figures and predicts that consumption will be 390,000 million kWh in 1990; but it still admits that there will be overcapacity. EDF argues that surplus electricity can be exported — French electricity is the cheapest in Europe, largely because of government pricing policy — and that the nuclear power industry must build at least two stations a year until 1990 or it will collapse. A successful nuclear industry will lead to nuclear exports later, the argument goes. But if nuclear power station construction continued at the present rate of four stations a year, it is estimated that coal burning for electricity — which makes up half the French coal market — would fall from its present level of 64,000 million kWh to 32,000 million kWh in 1985 and even less in 1990.

With little union backing for coal (the communist CGT union, which is strong in both the coal and nuclear industry has opted for nuclear energy), and with Chevènement behind nuclear power, it seems likely that the EDF view will be supported, and a substantial nuclear

programme maintained, with unpleasant consequences for the socialist party in the French coal districts in the municipal elections in the spring.

Why then are the forecasts falling? The answer is twofold. First, French economic growth is well below target, and seems unlikely to rise before 1985; and second there is growing confidence in the Agency Française pour la Maitrise de l'Energie (AFME), which seems to have had considerable success since its formation earlier this year from a group of disparate bodies concerned with energy conservation and renewable energies.

In 1983, AFME will invest FF1,200 million (£100 million), much of it in investment support grants which will raise a total of FF20,000 million (£1,300 million) in conservation and alternatives next year. The aim is to reach FF40,000 million (£2,600 million) a year by 1990, comparable with the present French investment rate in nuclear power. Some of the seed money is being raised by a tax on petrol at the pump.

By 1990, AFME is expected to have led to renewables usage of some 12 million tonnes of oil equivalent a year (around 10 per cent of primary energy consumption) with the greatest portion coming from wood and biomass, then from low-temperature geothermal heat (particularly by better exploitation of sources in the Paris basin) and finally from solar power. But the great saving comes in energy conservation — around 40 million tonnes of oil equivalent a year by 1990, according to AFME projections, which are broadly accepted by the Commission du Plan.

Robert Walgate

Genetic engineering

Human insulin

Washington

The commercial potential of genetic engineering was boosted in the United States on 29 October when the Food and Drug Administration (FDA) gave marketing approval to Humulin, a human insulin made with genetic engineering techniques. Made by Eli Lilly Company, the substance had won approval for marketing in the United Kingdom only a month before (see *Nature* 23 September, p.293). Although the announcement has symbolic value in the field and in particular for Genentech Inc., the California company that invented it, it will probably not quickly make a material difference to the fortunes of either Genentech or Eli Lilly.

Genetically-engineered human insulin is expected to be almost twice as expensive as the insulins now derived from pigs and cattle, which cost an estimated 26-30 cents per day of treatment. Lilly already has an estimated 85 per cent of the insulin market in the United States, so there is not likely to be a large market for Humulin soon, although the new product has an advantage in the few diabetics who develop an allergy

to animal insulins.

The announcement of FDA approval had been so long expected that neither Genentech's nor Lilly's stock jumped much in response, although Lilly's stock, which has been depressed since its sudden withdrawal of the anti-arthritis drug Oralflex earlier this year, traded a little higher after the FDA announcement on Humulin.

FDA gave approval on the basis of safe performance in trials involving about 400 patients a mere five months after Lilly filed its application. The agency also has in the pipeline applications for genetically-engineered products such as human growth hormones and interferons. Approval can take as long as two years, but the agency has recently undertaken a series of controversial moves to shorten approval time and simplify procedures.

Genentech is the first of the highly competitive biotechnology companies with a product approved for human use. The company's task was enormously eased by the security of the support provided by Lilly, which has exclusive licensing rights and which is estimated to have spent \$100 million in bringing the product to market. Genentech will receive a royalty on sales which is thought to be in single figures.

Genentech may be following a riskier path in its development of human growth hormone products and gamma interferon. It plans to market these products itself, which explains why it persuaded a group of investors to buy limited partnerships in October, thereby raising \$55 million for research. This will support work on a human growth hormone product for children who suffer from dwarfism, but since these are few in number the product would also have to be useful in combating CDSS (constitutionally delayed short stature), a problem that afflicts a greater number of people. Ideally, such a product could also help patients with cachexia, a wasting syndrome.

In addition to the limited partnership, in October four Swedish organizations bought \$20 million in Genentech's outstanding shares, or 571,428 shares at \$35 per share. (Two weeks later, after the limited partnerships and the FDA announcement, the company's stock reached \$50 a share.) The four organizations are Alfa-Laval AB, the Wallenberg Foundation, AB Fannyudde, an investment company affiliated with Volvo, and D. Caregie and Co., another investment company. The president and chief executive officer of Alfa-Laval AB, Harry Faulkner, was made a member of Genentech's board of directors. The company also appointed to its board John T. Potts, chief of the Department of Medicine at the Massachusetts General Hospital. Dr David W. Martin, a professor of medicine and biochemistry at the University of California, San Francisco, has been appointed vice-president for research.

Deborah Shapley

Biotechnology index

Biotechnology index beats Dow

Washington

Nature's third monthly Biotechnology Index set a new high in October, reaching 140.2 from a base of 100 at the end of June. This increase was greater than either the recent increases in the Dow Jones Industrial Index, the leading index of US industrial trading, or the Standard & Poor's 400 index of leading industrial stocks. Thus, the biotechnology companies moved parallel to, but ahead of, the market surge that led the Dow Jones index finally to break through the 1,000 mark in the first week of October.

But the performance of the *Nature* Biotechnology Index may have as much to do with internal factors in the companies themselves as with trends on Wall Street. The "Street" was reacting to continued lowering of inflation and the perception that the US elections, just held, would not upset the direction of the US economy. As one analyst in New York explained, the overall market surge was caused by "institutional money managers getting in who don't want to miss more of the rally". Such managers buy huge blocks of stock and so stay away from smaller companies

where such buying would artificially drive up the price.

The analyst went on to suggest that one reason why the *Nature* index did so well was because two companies in the index are large enough to attract some institutional funds and stable enough to ride along with the trend. These are A.B. Fortia of Sweden and Novo Industri A/S of Denmark, which had market capitalizations valued at \$751 million and \$789 million respectively on 29 October, when the index was calculated. The reasons for the rise in stock price of Genentech are outlined in the preceding story.

The other small companies in the index have also done well lately. "There have been no major flubs in the group", one analyst says. For example, the stock of Cetus, in Berkeley, California, rose after the company announced lay-offs and a re-direction of its research. This was seen as a net gain for the company, because specialists in biotechnology investment had felt that Cetus was over-extended. Hybritech and Damon of Needham have been getting considerable public attention for their work.

Deborah Shapley

Nature index of biotechnology stocks

1982 high	1982 low	Company	Close previous month	Close 29 Oct.	Change
45	16 1/8	A.B. Fortia (Sweden)	34 1/4	39 1/2*	5 1/4
8	2	Bio Logicals (Canada)	2 1/2	2 3/4	-1/4
7	3 5/8	Bio-Response (USA)	4 3/4	5	1/4
14 1/8	7 3/4	Cetus (USA)	8 3/4	9 3/4	1
11	6 1/8	Collaborative Research (USA)	8 5/8	9 3/4	1 1/8
9 7/8	5 3/4	Damon (USA)	7 1/2	9 1/8	1 5/8
18	8 1/4	Enzo-Biochem (USA)	14 1/2	17	2 1/2
28	6 5/8	Flow General (USA)	10 7/8	13 1/4	2 3/8
47 3/4	26	Genentech (USA)	33 3/4	47 3/4*	14 1/2
9 1/2	7	Genex (USA)	—	9 1/4	—
5 1/8	2 1/4	Genetic Systems (USA)	3 3/4	5 1/8	1
26 1/4	9 5/8	Hybritech (USA)	13 1/2	20 1/2	7
11 3/4	5	Molecular Genetics (USA)	10 3/4	9 3/4	-1
52	34 7/8	Novo Industri A/S (Den.)	44	47 1/2	3 1/2
17	8	Monoclonal Antibodies (USA)	8 1/4	14 3/4	6 1/2

The *Nature* Biotechnology Index for October, 1982, stands at **140.2**, compared with **117.9** last month. Base is 100 as of 25 June 1982. Previous indexes appeared in *Nature* 12 August, p.599; 9 September, p.101; and 14 October, p.573. Close-of-month prices are the closing prices on the last Friday of each month. Where stocks are traded over the counter, the price quoted is the bid price. For stocks traded on the American and New York Stock Exchanges, the price quoted is the transaction price. Data from E.F. Hutton, Inc.

*High or low for this calendar year.

In response to readers' comments on the Biotechnology stock index, its composition has this month been changed. Thus Collagen Inc. — a company that does not use new biotechnology — has been replaced by Genex, an important small company which made its first public stock offering after the close of the previous month's index.

This has been done by deleting Collagen's share of the market value of all the companies on 29 October and reallocating that share proportionally among the remaining 14 companies in the index. Then the total market value of Genex shares on that date (an estimated \$106 million) was added to the market values of the 14 companies and a new set of market shares calculated. This new set of divisors will be used in the calculation of future indexes.

In response to a further suggestion, there follows information on the trading symbols and, where appropriate, the stock exchanges on which the shares are traded. Most shares are traded over the counter (through brokers). Fortia (FOPHY); Bio Logicals (BIOLF); Bio-Response (BIOR); Cetus (CTUS); Collaborative Research (CRIC); Collagen (CGEN); Damon (DMN — NYSE); Enzo-Biochemi (ENZO); Flow General (FGN); Genentech (GENE); Genetic Systems (GENS); Genex (GNEX); Hybritech (HYBR); Molecular Genetics (MOGN); Novo Industri A/S (NVO — NYSE); Monoclonal Antibodies (MABF).

CORRESPONDENCE

Ethics of trials

SIR — The article on the proposed trials of the Medical Research Council (MRC) to investigate supplements in the prevention of neural tube defects (NTD) refers to ethical questions (*Nature* 16 September)¹. The MRC trial protocol conflicts with the revised Helsinki Code adopted by the 29th World Medical Assembly in Tokyo in 1975².

Dr Nicholas Wald is quoted as believing that a trial is ethical if "the likely good of the treatment" balances "the possible harm", implying that harm to one set of patients can be offset by benefit of another set, or of society. The revised code says (paragraph 1.5): "Concern for the interests of the subject must always prevail over the interests of science and society". Informed consent does not affect this obligation (paragraphs II.6 and II.4).

The judgement in the Nuremberg trial of doctors for unethical human experimentation in 1947 said: "The protagonists of the practice of human experimentation justify their views on the basis that such experiments yield results for the good of society"³. This same judgement enunciated the Nuremberg Code which was replaced by the Helsinki Code of the World Medical Association in 1964, revised in 1975.

Paragraph II.3 of the revised code says: "In any medical study every patient — including those of a control group, if any — should be assured of the best proven diagnostic and therapeutic method". The MRC trial is proposing to supplement a random half of patients with folate regardless of whether they are folate deficient, although techniques for assay of folate status are available and folate deficiency can be remedied in most patients.

Paragraph I.1 requires all research involving human subjects to be preceded by "adequately performed laboratory and animal experimentation". Folate deficiency in animals is reported to cause fetal resorption, low birthweight, small litter size, stillbirths and polymorphic malformations involving all body systems, as would be expected from folate's biochemical role in DNA synthesis. Folate deficiency delays development of the central nervous system in young children and is associated with mental illness in adults⁴. Paragraph I.9 requires subjects of human experimentation to be informed of "potential hazards of the study".

Folate is only one of over 30 nutrients known to be essential for normal embryonic growth. The comparative importance of different nutrient deficiencies in increasing the risk of malformations will only be discovered by extensive biochemical measurement of nutrient status. Randomized trials will never produce the answer if only because trials of 30 different nutrients at only two different levels of supplementation would require 3³⁰ trials. It can be argued that the MRC trial, because it will give no information on the prevalence of any nutrient deficiency, contravenes paragraph I.4 of the code which requires "the importance of the objective" to be in proportion to the inherent risk to the individual. The £300,000 would be better spent on well-planned biochemical measurements of the tissues or blood of women who have had babies with neural tube defects or some other malformations.

The MRC trial protocol contravenes paragraph I.12 of the code which says:

"The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with."

ARTHUR WYNN

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1. *Nature* 299, 198 (1982).
2. *World med. J.* 24, 76 (1977).
3. United States V. Karl Brandt in *Experimentation with Human Beings* (ed. Katz, J.) 305-306 (Russell-Sage, New York, 1972).
4. Botez, M.I. & Reynolds, E.H. (eds) *Folic Acid in Neurology, Psychiatry, and Internal Medicine* (Raven, New York, 1979).

CAB taxed

SIR — Many eyes must have been rubbed (and job applications drafted) when they read that employees of the Commonwealth Agricultural Bureaux no longer pay income tax (*Nature*, 14 October, p.572), and less self-interested souls must have wondered about the benefit of this to CAB member governments. The benefit lies in what amounts to a 30 per cent cut in taxable salary for CAB employees. A tax consultant works out what employees would normally pay in tax to the UK government, then hands this amount back to CAB. Take-home pay is thus virtually the same as before CAB's internationalization.

ALLAN ROSTRON

Edinburgh, UK

First to use?

SIR — In the article entitled "Vatican and the bomb" (*Nature* 4 November, p.2) you imply that the group convened by the Pontifical Academy in September called for a "declaration of no first use" of nuclear weapons. This is not true.

An earlier draft of the document, prepared by a group of which I was not a member, did indeed "call upon all nations to pledge never to be the first to use nuclear weapons". Many of those present at the final meeting, including myself, objected strongly to this wording on precisely the grounds you state in your article. The words "to pledge" were therefore omitted from this passage in the final document. This was signed by all the participants, and they included all but one of the fourteen who had prepared the earlier draft.

The final version of the document does not contain any suggestion that nations should make a "declaration of no first use" of nuclear weapons.

SIR ANDREW HUXLEY

President of the Royal Society

The Royal Society,
London SW1, UK

The distinction is important but not, without this explanation, readily apparent.

EDITOR, *Nature*

No to boycott

SIR — In responding to Dr Rowan-Robinson's appeal to end British participation in astronomy in South Africa (*Nature* 14 October, p.574) I must first declare an interest. I was one, if not the first, to propose a unification of observatories in South Africa at a single site at Sutherland and a leading activist in site selection and site testing. I quit British employ in South Africa — with the greatest regret — as Dr Rowan-Robinson would evidently wish others to do, in the expectation that judging by the contemporary official climate I might be deprived of observational opportunities. The establishment of the observatory at Sutherland was a subsequent reversal of policy. Sutherland is a very good site, comparable with those in Chile and superior to those in Australia. Although less well equipped than Siding Spring it offers to the observer relatively long runs at telescopes. Speaking from an experience of 30 years in telescope scheduling, I am certain that these are very often more productive, pound for pound and night for night than short runs on larger telescopes and permit greater flexibility in the choice of programmes. The facilities afforded by the site would be very hard to replace.

As to the question whether British participation condones or encourages apartheid this is more complex than would appear at first sight. Many of the actions of the South African Government are abhorrent to the international community. Boycotts of the type proposed are usually ineffective. Indeed some of the extreme right wing of the Nationalist party might be only too glad to see a diminution of the cultural influence of the English language and traditions. Such a boycott would primarily be a blow to a considerable section of the population who wish to see significant political changes. The South African Government does have a genuine problem of terrorist activity — presumably largely inspired by the Soviet Union which seeks to replace one tyranny by another and to gain control of the crucial oil route round the Cape and the strategic minerals of which southern Africa is the only source available to the free world. It is the hope of all reasonable men that political change will come peaceably and that many thousands of innocent and hard working people will not be sacrificed in salving the consciences of those remote from the scene of action.

I believe that the best hope for a peaceable solution is to increase rather than decrease cultural links from abroad. In a small way the present influx of scientific visitors promotes this. As to Sutherland itself, it is a dreadful location for the coloured people (there are almost no Bantu in the area) but at the observatory there are six coloured employees with good jobs and good housing. This is not much but it is a positive step towards ameliorating the lot of one section of the population which as far as I could judge from a recent visit is enjoying a period of striking economic advance.

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NEWS AND VIEWS

Anatomy of a human cancer gene

A difference of just one nucleotide distinguishes a human cancer gene from its normal counterpart. All of cancer is not that simple but the molecular biologist's approach should be reinforced.

The pages that follow contain an account of one of the most startling discoveries so far in the long and frustrating search for an understanding of cancer. The discovery is startling because it provides an unexpected answer to a question that has been on many people's minds since March last year¹ and certainly since this June^{2,3} — what is the difference between a cancer gene and its benign, physiologically indispensable version to be found in normal cells? This week we publish (on pages 143 and 149) two independent but essentially similar answers to this question from two groups in the United States, Dr Robert Weinberg's group centred on the Massachusetts Institute of Technology and Dr Mariano Barbacid's group at the National Cancer Institute. (Further independent confirmation is already in the publication mill.) And on the two following pages, readers will find a cool but enthusiastic appraisal of the significance of these discoveries from Drs Jonathan Logan and John Cairns, of Harvard University.

The essence of what Weinberg, Barbacid and their colleagues have shown is that in cells derived from a bladder carcinoma, there is a gene which differs from the naturally occurring gene in the simplest possible way — the replacement of one specific nucleotide in the DNA by another. The consequence is that the protein whose assembly is governed by the gene differs from the normally manufactured protein again in the simplest possible way — the twelfth amino acid (measured from the end of the protein molecule carrying a free amino group) is changed; in the bladder carcinoma cells studied, the normal glycine is replaced by valine. Nothing could be simpler.

So why the surprise? The most obvious explanation is that this simple result fits oddly with the opinion that has grown up in the past several years that because most kinds of cancer appear to involve cells that have somehow escaped from the unknown constraints that normally limit the multiplication of differentiated tissues, genetic explanations of cancer would probably turn on some property of the mechanisms by which the activity of various genes is controlled. Indeed, Weinberg and his associates describe how, in their study of what are known as the EJ bladder cancer cells, they first looked for differences from the normal in the region outside the gene proper where controlling elements are usually found. They found none of significance. Their account of how they turned to look for differences within the part of the gene which regulates (through the intermediary of messenger RNA molecules) the assembly of amino acid units into protein molecules is as nice an illustration as there could be of how logic can triumph over incredulity.

Several questions now arise, not the least of which is how such a small difference in the structure of a protein molecule can be responsible for such profound physiological differences in the characteristics of a cell, its conversion from a normal to a malignant cell. The fact that the protein concerned is known only as p21 is to be taken as a sign that little more is known about it than that it has a molecular weight of 21,000. The protein appears to be an enzyme and appears bound to the inner surfaces of plasma membranes (so that the production of an abnormal protein would not be apparent from the outside of a cell even if the abnormality were much more pronounced than that now found). Both the groups now reporting draw attention to the way in which a single amino acid substitution could nevertheless have a profound effect on the activity of an enzyme — Barbacid and his colleagues have used computer techniques to show that the replacement of the

small glycine unit by the much larger valine could have the effect of stiffening the end of the molecule, perhaps profoundly changing its catalytic properties in the process. However this may be, one thing is certain — there will now be mounted a determined effort to throw light on the normal function of the p21 protein, and of the other proteins it may influence. That will be a gigantic undertaking.

Even at this stage, however, it is clear that the discovery now reported is not a universal explanation of cancer. Thus Barbacid and his colleagues point to the apparent contradiction between their discovery and the demonstration earlier this year² that it is possible to make normal cells cancerous simply by infecting them with DNA corresponding to the normal p21 gene to which has been attached pieces of viral DNA corresponding to those thought to be necessary for the functioning of the tumour viruses known as retroviruses. The result of that procedure is to increase the amounts of p21 produced, which suggests that the amount of the protein generated is also relevant. A little abnormal p21, or a lot of the normal product, seems to have similar consequences for a cell, but for reasons not yet properly understood. And why should there be such a close relationship between the cancer genes now described and the retroviruses? Logan and Cairns point in what follows to the fascinating questions raised by that train of speculation.

So where will it all end? Logan and Cairns rightly point out that the discoveries now reported cannot conceivably amount to an explanation of the general phenomenon of cancer. For decades, it has been all too clear that both the causation and the course of different kinds of cancers are exceedingly varied. The importance of what has now been done is that it points unambiguously to one feature of the biochemical apparatus of the vertebrate cell whose abnormality must be supposed to be critical for the development of malignancy. Inevitably there will now be a hunt for a similar analysis of the other known cancer genes, close on a dozen of which are known. It is in the literal sense marvellous that the techniques of genetic manipulation developed in the past decade should now be used to such good and delicate effect (which is in no sense to belittle the difficulty of what has been accomplished, as a reading of the two articles, each with its interlocking network of individual experiments, will quickly show).

What has happened, as Logan and Cairns point out, is certain to change the way in which cancer research is planned. In recent decades, there has been recurrent tension between those (like Cairns himself⁴) who argue that the prime objective should be to understand the phenomenon of malignancy and those who hold that the problem is so complicated that it would be better to concentrate on identifying the causes of cancer — and then to avoid them. Nobody will now pretend that understanding is so close at hand that more empirical ways of avoiding cancer or tackling the consequences can be abandoned, but there is the strongest case for seeing that the huge sums of money available for research on cancer are redirected in the directions to which these new discoveries point, and that they are not exclusively spent by the large laboratories but, rather, by those groups of workers whose flair and versatility seem finally to have put paid to the old slur that molecular biology has done nothing for patients.

1. Shih, C., Padhy, L.C., Murray, M.J. & Weinberg, R.A., *Nature* **290**, 261-264 (1981).
2. Chang, E.H., Furth, M.E., Scolnick, E.M. & Lowy, D.R. *Nature* **297**, 479-483 (1982).
3. Parada, L.F., Tabin, C.J., Shih, C. & Weinberg, R.A. *Nature* **297**, 474-478 (1982).
4. Cairns, J. *Nature* **289**, 353 (1981).

The secrets of cancer

from Jonathan Logan and John Cairns

THIS year may one day be seen as the year when a strong sense of order came to cancer research and the long drive to understand the cancer cell really got under way. To appreciate the significance of papers like the ones appearing in this issue (Tabin *et al.* p.143 and Reddy *et al.*, p.149) we have to look back at some recent history.

Molecular genetics has brought to biology a new level of order and understanding. The informational macromolecules have been identified and we can now trace at least some of the simpler programmes in metabolism back to their source in the logical circuitry of DNA. What made this possible, more than anything else, was the discovery that genes could be transferred from one cell to another. Without bacterial transformation and the special transfer of information that occurs during virus infection, it would have been hard to show that genes are made of DNA. Without bacterial mating and transduction, dissection of the operon and its component parts would have been almost impossible. Given the wisdom of hindsight, we can see that even the most detailed catalogue of the changes that occur when *Escherichia coli* is fed lactose could not, by itself, have told us how cells regulate protein synthesis. Fortunately, the constraints imposed by evolution require genes to be capable of functioning in almost any context. In the bacterial cytoplasm, everything can interact with everything else, and trying to analyse it is like trying to understand the economy. In the genome, however, genes battle it out one by one, although they are of course being judged by their effects upon the cytoplasm. It is this wonderful separation of living systems into one arena of great complexity and another of great simplicity that has made it possible to find out how cells know what they ought to be doing.

This message has not yet had its full impact on cancer research. A glance at the current journals will show how many people are still trying to understand the biology of cancer by making inventories of the properties of the cells present at each stage of carcinogenesis. Of course, the transition has been difficult. To anyone accustomed to the delights of bacterial genetics, the vertebrate cell must seem an impenetrable forest, with its vast genome, poorly understood systems for gene regulation and — most important of all — its relative inaccessibility to the techniques of genetics. Nature, however, provided one avenue of approach, the tumour viruses. Despite the slender means at their disposal (a few genes-worth of nucleic acid) they possess the means to make a cancer. This fact alone tells us that the various systems of control in animal cells come together at certain focal points ('nodes' in computer

terminology) where they can be subverted by single genes and their unique gene products. As we shall see later, evidence is now emerging that these focal points can be the site of attack for at least two other completely distinct forms of carcinogenesis.

Until recently, the vast size of the mammalian genome effectively prevented any direct search for the cellular genes involved in cancer. Without some selective procedure, searching for a mammalian gene is equivalent to looking, without an index, for an article of this length (2,000 words or 20,000 characters) that is known to have appeared in *Nature* some time since 1900 (that is, somewhere in a text of 3×10^9 characters). The genes were discovered, however, because they confer tumorigenicity when they are picked up by certain retroviruses: the virus was therefore doing the selection and the purification. The genes have come to be called 'oncogenes' as if their primary role were to cause cancer. But it was soon realized that typically they are cellular genes ('proto-oncogenes'), whose function is sufficiently important for them to have been highly conserved over the long period of evolution of the vertebrates. One of them is known to change its level of expression during embryogenesis and so it may be a gene normally concerned with development. Another has been shown to code for a protein kinase capable, in principle at least, of modulating the properties of many different gene products, which is just the sort of operation we might expect to find at a focal point in the regulatory circuits of the cytoplasm. But the function of most of the dozen or so identified oncogenes is not yet known. Presumably, each oncogene is not equally important in every tissue and therefore will be more often involved in some kinds of cancer than in others.

The tumorigenic retroviruses can cause cancer in at least two different ways. The virus can bring in an oncogene which, because it is under the control of the viral promoter of transcription, is inevitably transcribed at too high a level. Alternatively, the virus need not contain an oncogene, but merely provide a strong promoter which, by chance, can then become integrated next to one of the cell's oncogenes; this is a much less efficient form of tumorigenesis and usually succeeds only after a long time interval. To summarize, the product of each cellular proto-oncogene seems to be capable of making a cell into a cancer provided that it is produced to excess or has undergone a change in amino acid sequence.

Because the retroviruses are so adept at picking up, transporting and reintegrating cellular genes, they have drawn attention to the particular genes that can endow them

with tumorigenicity. It could therefore have been argued, at least until recently, that none of this has much to do with human cancer which, as far as we know, is seldom brought on by an RNA retrovirus. In the past ten years, however, a completely different way has been found for studying the genes involved in cancer. Certain cell lines (most notably the NIH line of 3T3 mouse fibroblasts) have been found to be capable of taking up raw DNA and to be transformed to permanent tumorigenicity if the incoming DNA has been derived from animal, or human, tumours or tumorigenic cell lines. Since human DNA contains certain telltale sequences, not found in mouse DNA, it has been possible (given the formidable armoury of restriction enzymes and all the other paraphernalia of genetic engineering) to isolate these human genes that are tumorigenic for NIH 3T3 cells and presumably were at least partly responsible for the cancers from which they were derived. The fact that such experiments are possible is, in itself, a dramatic demonstration that alteration of the genome is an essential step in the production of some cancers. At this point, it is simplest to list the other major conclusions to have come from these studies of 'transfection'.

First, it is just possible, with great difficulty, to transform NIH 3T3 cells by feeding them their own DNA, provided that it has been fragmented into roughly gene-size pieces; but these transformed cells are then an efficient source of transforming DNA, provided that it is not highly fragmented. By analogy with the retroviruses, it seems likely that the first, and difficult, step is to bring one of the cell's proto-oncogenes under the control of one of the cell's strong promoters, but once these have been brought together they can easily transform other cells, provided that they are not separated.

Second, when efficiently transforming DNA arises in naturally occurring tumours, or is found in retroviruses, it can function across species barriers; for example, human tumour DNA can transform mouse cells. This is what we might expect, since the oncogenic sequences have been so closely conserved during evolution.

Third, in several instances, the tumorigenicity of DNA from human tumours has been traced to the activation of one of the oncogenes that had been identified in murine sarcomas (either the Harvey or the Kirsten *ras*), suggesting that a change in the function of either of these can be a step in the formation of cancer in several organs. Interestingly, in the examples studied in this issue of *Nature*, the tumorigenicity of DNA seems to be

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due, not to a change in level of expression of the oncogene, but to a change in its coding sequence; this suggests that the cancer may have resulted, in part, from exposure to the kind of mutagen that can produce this particular change, a GC to TA transversion.

We may be seeing here the shape of things to come. Even if mutation of the oncogene was not actually one of the rate-limiting steps in that particular patient's progression to bladder cancer, it does seem that the requisite technology is fast approaching that will make it practicable to search through selected regions of the genome for every kind of textual aberration. One spectacular example of this is about to be reported elsewhere (P. Leder, personal communication). Most B-cell lymphomas bear a translocation of the end of chromosome 8 into the region of one of the chromosomes concerned with antibody synthesis (chromosomes 2, 14 or 22). It has now been possible to isolate the region of DNA involved in this rearrangement and show that it contains the proto-oncogene called *myc*, that was originally found in association with a chicken leukaemia and, in humans, is located on chromosome 8. So here too is another human cancer arising apparently as the result of activation of an oncogene, in this case because the gene has become caught up in the active chromosomal region involved in antibody synthesis.

We seem, therefore, to be at one of those wonderful moments in science when many lines of research come together and much that was impossibly obscure suddenly seems plain. There are, however, one or two caveats that are well known to everyone engaged in this work but may not be immediately obvious to outsiders.

Natural carcinogenesis is a multistep process. Evidence for this comes from epidemiology (for example, the relation

between incidence of lung cancer and the frequency and duration of cigarette smoking) and from laboratory experiments, and shows that the formation of a cancer requires several independent, rate-limiting steps. Thus, the provision of an active oncogene, whether by activation of a cellular gene through rearrangement or mutation or by viral infection, presumably corresponds to only one of a sequence of steps. Given that cancer arises from a sequence of steps, we would expect the first steps to be the ones that allow clonal expansion and free competition between cells, because this should then make all the other steps occur more efficiently and allow continuous selection of faster growing cells. But special provisions appear to have been made to prevent competition between individual cells in intact tissues. So a significant component of carcinogenesis in whole animals must be the production of whatever changes are needed in order to allow competition. If, for example, the first step in carcinogenesis has to be the liberation of cells from control by cell-cell interaction, we could not find this out by studying the transformation of cell lines which have, of course, already taken this step.

Transfection experiments with NIH 3T3 cells are open to other objections. Mice will develop cancer at the site of implanted plastic sheets, and it may be for this reason that their cells can readily be induced to grow profusely in tissue culture dishes. Guinea pigs (and probably humans) are not susceptible to such foreign body carcinogenesis, and their cells do not readily form immortalized cell lines. We could argue that the 3T3 cell is already part-way to becoming a cancer cell, and for that reason may not provide the right assay for certain kinds of tumorigenic DNA.

In transfection experiments only about one in a million cells acquire and express

the exogenous DNA. This may mean that only cells already possessing a special genetic or metabolic alteration are competent to acquire foreign DNA. Such cells could be just those that have taken one of the rate-limiting steps towards cancer; if transfectibility is evidence of a step towards transformability then this step cannot be elucidated in these experiments. (Although transformation by viruses is very efficient, the viral reverse transcriptase could conceivably be making gene rearrangements much more frequent and, in that way, be by-passing what is normally a rate-limiting step.)

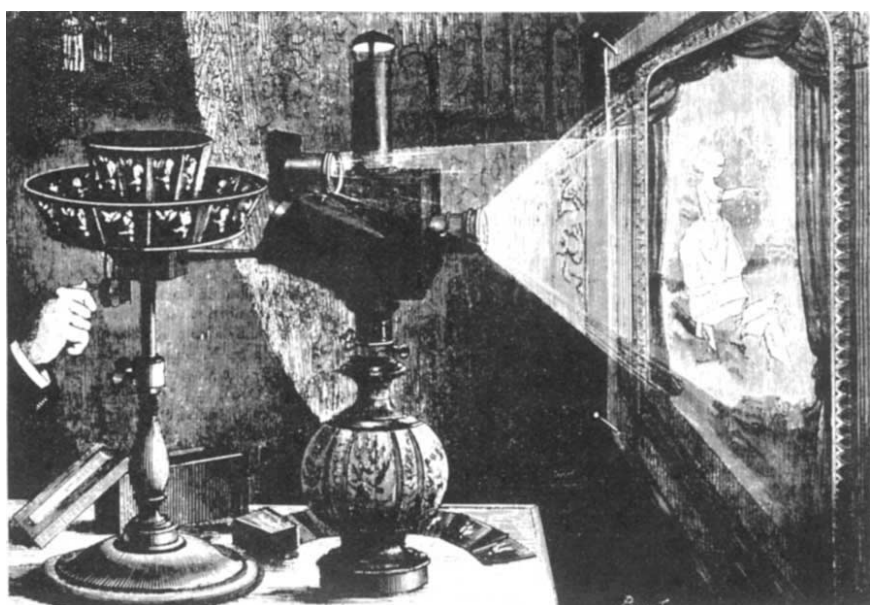
Most tumours studied so far, whether occurring naturally in humans or produced by X rays and chemicals in animals, do not yield DNA that is oncogenic in cell transfection experiments. So the assay, as presently constituted, is not detecting the genetic events responsible for many types of cancer. It is important to remember that transfection can only pick up changes that, in the genetic sense, are dominant; also it will only detect oncogenes which provide products that these particular recipient cells require for transformation (for example, the *myc* gene is not efficient at transforming NIH 3T3 cells); lastly, there is the requirement in these experiments (and in tumorigenesis by viruses) that the transforming genes are not too large to be transferred intact.

Above all, we need to keep in mind that natural human carcinogenesis is a multistep process. We do not know what the rate-limiting steps are, nor which of them are most sensitive to environmental influences and therefore a proper object for preventative strategies. But we can at least feel that the technology for studying genes and their patterns of expression are now advancing so fast that the molecular biologist will soon be telling the epidemiologist what to look for. □

100 years ago

THE PROJECTION PRAXINOSCOPE

M. GASTON Tissandier describes in *La Nature* an ingenious adaptation of the praxinoscope, under the above name, by means of which the images are projected on a screen, and are visible to a large assembly. Our engraving will give an idea of the arrangement and the effect produced. By a modification of the "lampascope", M. Reynaud, the inventor, obtains by means of an ordinary lamp, at once the projection of the scene or background — by the object-glass which is seen at the side of the lantern — and of the subject, by another object-glass which is shown in front of and a little above the same lantern. For this, the positions of phases which form a subject are drawn and coloured on glass, and are connected in a continuous band by means of any suitable material. One of these flexible bands is placed in the wide crown of the praxinoscope, which is pierced with openings corresponding to the phases of the subject. A hand-lever on the foot of the instrument allows a moderate and regular rotation to be communicated.



From *Nature* 27, 61; November 16, 1882.

The Nobel prize in physics

from Norman Dombey

THE award of the Nobel prize in physics to Ken Wilson of Cornell University honours the first of a new generation of theoretical physicists who are as familiar with the physics of thermodynamic phase transitions or the first second of existence of the Universe as they are with electron-proton scattering. This trend marks a welcome return to the spirit of the 1920s and 1930s, when the pioneers of quantum mechanics were equally at home with crystal lattices, molecules, atoms and the recently discovered nuclei. Wilson is not the only theorist to have noticed similarities between the theoretical analysis of renormalization in relativistic quantum field theory (which describes the behaviour of elementary particles) with the then unsolved classical problem of the universality of the critical indices of phase transitions between differently ordered macroscopic states of a system (such as the liquid-gas transition), but no other physicist has had the insight and stamina necessary to see the work through and to develop a general method for the calculation of critical indices.

A ferromagnet is a good example to illustrate universality. In ferromagnets, spontaneous magnetization occurs — magnetization persists in the absence of an external magnetic field, provided that the temperature is less than some critical temperature T_c . It is found experimentally that magnetization goes to zero as the temperature T tends to T_c as a power β of $(T - T_c)$. β for all ferromagnets has a value between 0.3 and 0.4. Similarly, other quantities, such as magnetic susceptibility, vary near T_c as a power γ of $1/(T - T_c)$ and experimentally $\gamma = 1.2-1.4$. Although the Ising model of a ferromagnet (see *News & Views* 299, 675) was solved by Onsager in two dimensions to show that a phase transition exists, and can be solved numerically in three dimensions to give critical indices in reasonable agreement with experiment, it does not provide any explanation of the universality of behaviour near the critical temperature (which itself varies widely between ferromagnets).

The problem of universality can be phrased as follows: starting with an energy function describing the microscopic properties of the system of interest and containing the physical parameters of the system, for example the spin-spin interaction between neighbouring lattice sites, is it possible for the equilibrium state near the critical temperature to be independent of the initial parameters? If so, then the critical indices will depend only

on the gross features of the initial energy function, such as whether the physical variable involved is a scalar or vector quantity and whether the underlying space is two- or three-dimensional. Kadanoff² had shown that spin-blocking (the pairing and re-pairing of spins on the lattice) provided a recursive set of transformations which could in principle take the initial energy function into the form desired.

There is an analogous result in relativistic quantum field theory. The initial parameters in the Lagrangian (the appropriate relativistic energy function) are not the measured physical parameters. This is because infinities occur in the calculational procedures and in order to remove these infinities a process of renormalization is carried out whereby the infinities are subtracted out at some arbitrary momentum Λ . Hence the physical parameters of the theory, for example charge and mass, are functions of the initial parameters and Λ , but the results of any calculation must only depend on the physical parameters and not on Λ . So a change of scale whereby Λ is replaced by 2Λ should not have any influence on the result of a calculation. Gell-Mann and Low³ in 1954 wrote down the mathematical conditions necessary for this invariance and these are known as the renormalization group (RG) equations of the theory. Certain scaling laws among the field quantities and momenta of the theory follow from the RG equations. This is the problem that Wilson studied further throughout the 1960s.

Wilson is most remarkable in that during this period he was not at all interested in publishing papers. He received his PhD from Caltech in 1961 with a thesis⁴ on a problem in quantum field theory where a relativistic pion field interacts with a static nucleon. This problem is complex enough to be interesting while tractable enough for the RG equations and scaling laws to be worked out. He did not publish the thesis and he only published one paper⁵ on his progress on this problem until he obtained his main results which were published in a series of papers⁶ beginning in 1969; the simplified model of his thesis played a major part in the derivation of his results. These results, very approximately, show that in a renormalizable theory at sufficiently high momenta when all masses in the theory can be neglected and therefore no basic momentum (or length) scale exists, the limiting form of the physical amplitudes are given in terms of numbers called the anomalous dimensions of the theory which are calculable using the RG equations.

Michael Fisher, who had worked for many years on the numerical calculation of

critical indices, went to Cornell in 1966 from King's College, London and Wilson's awareness of the problem of universality probably stems from his presence. But it also stemmed from the field theory calculations of the fixed nucleon model in that in order to make progress with his integral equations, Wilson had split up the space-time continuum into cells, thereby giving it a lattice structure. Reversing this process, so that a continuum field theory limit could be taken starting from the crystal lattice of a ferromagnet, was a natural development.

In the region of a phase transition the correlation length in a material becomes arbitrarily large, so again there is no basic length scale. It did not take Wilson long to convert the Ising model into field theory and to look for the RG equations of the theory, albeit now in three dimensions rather than the four of relativistic field theory. For the phase transition he had to work in the low-momentum limit (long-wavelength) of the RG equations whereas in particle physics the high-momentum (short-wavelength) limit pertains. Nevertheless, anomalous dimensions were translated into critical indices and he was able in 1971 to publish a calculation of γ for the three-dimensional Ising model using the RG equations⁷. Techniques for the calculation of critical indices via the RG equations soon improved, largely due to a trick invented by Wilson and Fisher⁸, and it became apparent that universality did indeed follow from the RG equations⁹ since the initial parameters in the energy function were irrelevant near the phase transition.

Perhaps the most interesting lesson to be drawn from Wilson's career is that difficult problems in science deserve long study (otherwise they would not be difficult) and that solutions may well lie in methods developed in a different area of science. So extreme specialization (such as that of graduate students) and the compartmentalization beloved of bureaucrats (for example, in Britain ferromagnetism is dealt with by the Science Board of SERC while particle theory comes under the Nuclear Physics Board) may not be helpful. Another lesson is for tenure committees and for those social scientists who aim to measure successful science: it is not good enough to weigh publications or count citations. Truly original work is unlikely to

1. Larkin, A.I. & Khmel'nitskii, D.E. *Zh. Eksp. Teor. Fiz.* 56, 2087 (1969); *Sov. Phys. JETP* 29, 1123 (1969); Di Castro, C. & Jona-Lasinio, G. *Phys. Lett.* 29A, 322 (1969); Migdal, A.A. *Zh. Eksp. Teor. Fiz.* 59, 1015 (1970); *Sov. Phys. JETP* 32, 52 (1971).
2. Kadanoff, L.P. *Physica* 2, 263 (1966).
3. Gell-Mann, M. & Low, F.E. *Phys. Rev.* 95, 1300 (1954).
4. Wilson, K.G. *An Investigation of the Low Energy and the Chew-Mandelstam Equations*, thesis, California Institute of Technology (1961).
5. Wilson, K.G. *Phys. Rev.* 179, 1499 (1969); D2, 1438 (1970); D2, 1478 (1970); D3, 1818 (1971).
6. Wilson, K.G. *Phys. Rev.* B4, 3174, 3184 (1971).
7. Wilson, K.G. & Fisher, M.E. *Phys. Rev. Lett.* 28, 240 (1972).
8. For a general review, see Wilson, K.G. & Kogut, J. *Phys. Rep.* 12C, 75 (1974).

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be produced on a production line. Finally, on a hobby-horse of my own, universality, where it exists in physics, is a concept based on real experimental results, unlike the

present theoretical chimaera of unification for which there is as yet no hard experimental evidence (see *Nature* 279, 675; 1979). □

quence alterations and EC differentiation, however, is not clear, especially as the mutants grow normally on mouse fibroblasts. New mutants have also been isolated which are capable of infecting cell lines isolated from mid-term mouse placenta, these mutants also grow in 3T3 but cannot grow in EC cells (K. Tanaka, National Cancer Institute). At least some of the EC selected mutants can grow in the placenta-derived lines. Before these results can be ascribed to specific developmental changes it is necessary to know what cells, if any, in the adult are refractory to polyoma infection. The block to retrovirus expression is also poorly understood. The simplest explanation is that expression of virus introduced into EC or early embryonic cells (C. Stewart and R. Jaenisch, University of Hamburg) is prevented by methylation — other mechanisms must also be involved, though, for inhibition can occur before full methylation is detected (J. Gautsch, Research Institute of Scripps Clinic).

One of the exciting recent findings in molecular biology is the observation that the transforming genes of many oncoviruses have equivalents in normal cells⁹. The possibility therefore arises that they may be genes that are important for regulating the growth of specific cell types, and may themselves be developmentally regulated. Many cellular oncogenes are indeed expressed differentially in development and several are expressed in EC cell lines (R. Muller, Salk Institute). Some of the cellular oncogenes (or at least their viral equivalents) are protein kinases which phosphorylate tyrosine¹. It is thus interesting that several phosphotyrosine-containing proteins can be identified in early stage mouse embryos and EC cell lines and changes in them can be observed (C. Carlin, Wistar Institute).

Probably one of the most important technical innovations to be discussed at the conference was the recent discovery of a technique of deriving pluripotent EC cell lines from embryos explanted *in vitro* (E. Robertson, University of Cambridge; G. Martin, UCSF). At the earlier Nutley

The biology of teratocarcinomas

from P.N. Goodfellow and P.W. Andrews

THE early development of mammals is one of the major areas of biology not yet fully susceptible to the techniques of molecular biology. The chief obstacle is that the very fact of development makes it difficult to obtain enough cells, at the same stage of development, for molecular and biochemical analysis. Teratocarcinomas and their stem cells (embryonal carcinoma or EC cells) offer a solution, for these embryonic tumours display extensive cellular differentiation, often into derivatives of all three germ layers as well as the extraembryonic tissues. EC cells can be cloned and maintained *in vitro* as undifferentiated cell lines and differentiation can then be induced by altering the growth conditions, transplanting the cells into mice or by chemical inducers. Seven years ago a meeting on the biology of teratocarcinomas was held at Nutley, New Jersey¹ and progress since then was debated at a recent conference* held at Cold Spring Harbor.

Complex systems involving cellular interaction and the generation of diverse cell types can only be studied if the various cells in the system can be distinguished and if the changes in cellular phenotype are under the control of the investigator. Traditionally, morphology has served as the primary indicator of differentiation, but its assessment is subjective and it can be misleading. However, the number of biochemical and immunological markers of cellular differentiation in EC cells is rapidly expanding. These include cell surface antigens (for example, the EC marker, SSEA-1)², α -fetoprotein³, alkaline phosphates⁴, plasminogen activator⁵, the various cytoskeletal elements (R. Oshima, La Jolla Cancer Research Foundation; R. Kemler, Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft) and basement membrane components (C. Howe, Wistar Institute; B. Hogan, ICRF). Chemical inducers, such as retinoic acid, dibutyl

cyclic AMP and dimethylsulphoxide, permit induction of differentiation along pathways which are apparently specific for the inducer and the particular EC cell line used (M. McBurney, University of Ottawa; M. Damon, Pasteur Institute). The mechanisms by which these substances cause their effects is not known, although for retinoic acid there is an intracellular receptor, which appears to mediate the action of this agent (M. Sherman, Roche Institute of Molecular Biology, Nutley). Differentiation in culture seems also to be affected by cell contact^{6,7}, by growth factors produced both by stem cells (L. Gudas, Sydney Farber Cancer Institute) and by differentiated derivatives (C.M. Isacke, University of Oxford), and by interaction with extracellular matrices composed of basement membrane components such as laminin, fibronectin and type IV collagen. For example, organization of a laminin-containing basement membrane in embryoid bodies produced by suspension cultures of EC cells seems to be an important prerequisite for the outer cells to complete their differentiation to α -fetoprotein-secreting visceral endoderm-like cells (E. Adamson, La Jolla Cancer Research Foundation).

While definition of the ways EC cells differentiate in culture represents a first step, eventually it will become important to study the changes that occur at the molecular level. However, cloned DNA probes for genes regulated in early development are still a rarity. Those which are available suggest that regulation occurs, at least in part, at the level of transcription, as for the *H-2D,K* genes of the mouse histocompatibility system (G. Gachelin, Pasteur Institute). The cloning of further developmentally regulated genes is clearly a priority, and several groups are actively pursuing this area (K. Marotti, Rockefeller University; B. Hogan; C. Howe).

In the meantime, viruses remain valuable probes of cellular differentiation. Although mouse EC cells will not support expression of small tumour viruses such as polyoma or the C-type retroviruses, they can be infected by polyoma mutants which appear to be related by having suffered sequence rearrangements and mutations in the early promoter region (in the *PvuII-4* fragment⁸) (M. Vasseur, Pasteur Institute; E. Linney, La Jolla Cancer Research Foundation; J. Lehman, University of Colorado). The relationship between the se-

*Teratocarcinoma stem cells — the tenth Cold Spring Harbor Conference on Cell Proliferation was arranged by L. Silver (Cold Spring Harbor Laboratory), G.R. Martin (University of California, San Francisco) and S. Strickland (Rockefeller University).

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1. *Teratomas and Differentiation* (eds Sherman, M.I. & Solter, D.) (Academic, New York, 1975).
2. Solter, D. & Knowles, B.B. *Proc. natn. Acad. Sci. U.S.A.* 75, 5563 (1978).
3. Dziadek, M. & Adamson, E.D. *J. Embryol. exp. Morph.* 43, 289 (1978).
4. Bernstein, E.G., Hooper, M.C., Grandchamp, S. & Ephrussi, B. *Proc. natn. Acad. Sci. U.S.A.* 70, 3899 (1973).
5. Strickland, S., Reich, E. & Sherman, M.I. *Cell* 9, 231 (1976).
6. Martin, G.R. & Evans, M.J. *Proc. natn. Acad. Sci. U.S.A.* 72, 1441 (1975).
7. Hogan, B.L.M., Taylor, A. & Adamson, E. *Nature* 291, 235 (1981).
8. Tooze, J. in *DNA Tumour Viruses* 2nd edn (Cold Spring Harbor Laboratory, New York, 1981).
9. Cooper, G.M. *Science* 217, 801 (1982).
10. Hunter, T. & Sefton, B. *Proc. natn. Acad. Sci. U.S.A.* 77, 1311 (1980).
11. Brinster, R.L. in *Teratomas and Differentiation* (eds Sherman, M.I. & Solter, D.) 51 (Academic, New York, 1975).
12. Mintz, B., Illmensee, K. & Gearhart, J.D. in *Teratomas and Differentiation* (eds Sherman, M.I. & Solter, D.) 59 (Academic, New York, 1975).
13. Papaioannou, V.E., McBurney, M.W., Gardner, R.L. & Evans, M.J. *Nature* 258, 70 (1975).

meeting one of the observations reported was the ability of EC cells, transplanted into a blastocyst, to participate in normal embryonic development, leading to a chimaeric mouse with tissues derived from both the host blastocyst and the EC cell¹¹⁻¹³. However, the efficiency of chimaerism with most EC cell lines is low; this may be a result of small chromosomal abnormalities of many EC cells after prolonged *in vitro* culture, although other factors may be involved since some apparently euploid lines also fail to form chimaeras (J. Rossant and M. McBurney, Brock University, Ontario). Since the embryo-derived EC cells seem to have less chromosomal abnormalities, at least in early passage, the hope is that these may produce much higher rates of chimaerism.

The other important possibility stemming from this technical advance is that of readily introducing known embryonic

mutant genes into EC lines *in vitro*. For example, embryos homozygous for mutants of the *T/t* complex die at various stages of development. The mechanism of action of these lethal genes remains obscure, but suggestions varying from the aberrant expression of cell-surface molecules important in critical cell interactions to general metabolic deficiencies have been proposed. An embryo-derived EC cell line, homozygous for *t^{ws}*, which causes embryonic death at about day 8 of development, has been isolated (T. Magnusson, UCSF). However, this *t^{ws}/t^{ws}* EC cell line grows normally *in vitro* and forms well differentiated tumours when injected into mice. These observations suggest that embryonic lethality caused by this mutant is due to inability to organize embryonic structures at the proper time, rather than an inability simply to grow and differentiate. □

Bio derivatives can be introduced easily into RNA and DNA. Bio-dUTP is a good substrate for the enzymes DNA polymerase I of *Escherichia coli* and bacteriophage T4 DNA polymerase and can be incorporated efficiently into DNA by 'nick-translation'. Bio-UTP can be incorporated into cRNA by *E. coli* RNA polymerase. Unfortunately, the analogues do not seem to function as substrates for avian myeloblastosis virus reverse transcriptase or for eukaryotic RNA polymerases.

In hybridization, Bio-substituted nucleic acids behave similarly to the non-substituted versions. There is no change in the melting temperature of DNA containing 20 biotin molecules per kilobase introduced by nick translation. Viral DNA in which all the thymidines in one strand are replaced by Bio-dUMP shows a drop in melting temperature of only 5°C. Lightly substituted DNA (5.5 per cent of thymidine substituted by Bio-dUMP) hybridizes in solution at the same rate as non-substituted DNA. The substitution may have some useful side effects — the hydrophobic side chain added to the DNA changes its physical properties and, for example, results in it being extracted into the phenol phase during phenol extraction. This could cut down background during hybridization on either nitrocellulose filters or on glass-mounted cytological preparations.

Langer-Safer and colleagues (Langer-Safer, Levine & Ward *Proc. natn. Acad. Sci. U.S.A.* **79**, 4381; 1982) have now used the biotin-labelled DNA to detect specific sequences in *Drosophila* polytene chromosomes by *in situ* hybridization. Here there are no problems of detection as the DNA in the polytene chromosomes is replicated many times. The probes were prepared with between 8 per cent and 40 per cent of their thymidine residues substituted with Bio-dUMP, and after hybridization to the chromosomes they were reacted with rabbit anti-biotin antibody. For direct fluorescence detection the slides were incubated with goat anti-rabbit antibody labelled with fluorescein and examined by phase-contrast and fluorescence microscopy. There are a few disadvantages to this method, in particular bleaching of the fluorescent signal under the light source, so a second method, using a histochemical stain, was also employed. The slides were incubated with horseradish peroxidase-conjugated sheep anti-rabbit IgG and the peroxidase activity then used to convert the substrate diaminobenzidine into an insoluble brown precipitate which can be clearly distinguished in Giemsa-stained preparations. One valuable aspect of this method is that the length of chemical exposure can be continuously monitored to give an optimal reaction time. The same method has been used by Manuelidis to detect repeated sequences in human chromosomes and mouse satellite sequences in mouse chromosomes [in *Genome Evolution* (eds Dover, G.A. &

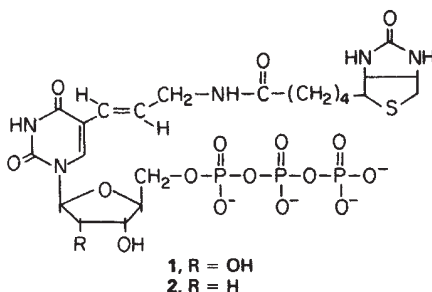
Lighting up time

from Sue Malcolm

THE report from David Ward's laboratory at Yale University School of Medicine of a new method for detecting specific DNA sequences suggests that a considerable increase in both sensitivity of detection and resolution may be just round the corner. The technique may be particularly valuable in the antenatal detection of those hereditary diseases where the molecular and chromosomal mapping of the genes involved has already been made possible. The basic idea is to incorporate a biotin-labelled nucleotide into the nucleic acid with an anti-biotin antibody (raised in rabbit) and then detect the complex either by fluorescence with a second antibody (goat anti-rabbit) coupled to fluorescein or cytochemically with the second antibody coupled to an enzyme such as horseradish peroxidase.

Present techniques rely on radiolabelling cloned DNA fragments with ³²P- or ¹²⁵I-labelled nucleotides. After hybridization, the labelled sequences can be detected with great sensitivity, but there are a number of limitations to the use of the isotopes. ³²P has a half-life of only two weeks and probes must be made and used immediately. This presents particular difficulties for laboratories any distance from the major suppliers (such as clinical laboratories in many of the countries where antenatal diagnosis of the haemoglobinopathies is desirable), and the handling and disposal of the radio-isotopes presents a health hazard, not to mention a bureau-

cratic hazard. The high energy of the radioactive emissions, particularly of ³²P, makes the grains on the film too scattered to give useful resolution in detecting specific mRNA sequences in individual cells, or genes on chromosomes. Tritiated nucleotides give much better resolution in cytological specimens but are necessarily also of lower specific activity, and the amount of nucleic acid corresponding to a single-copy gene on an individual chromosome or a low-abundance mRNA in a single cell is so



Biotin-labelled derivatives of UTP (1) and dUTP (2). In 1, R = OH and in 2, R = H.

low that their detection only becomes possible with the use of very long autoradiographic exposure times or the pooling of data.

The new technique makes use of biotin-labelled derivatives of dUTP and UTP (see the figure) synthesized via 5-mercaptopurine intermediates (Langer, Waldrop & Ward *Proc. natn. Acad. Sci. U.S.A.* **78**, 6633; 1981). Other nucleotides could be synthesized by the same method but dUTP and UTP have proved the easiest to make. The

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Flavell, R.B.) 263, Academic, New York; 1982].

The method has many potential variations, the most obvious being the use of avidin-bound enzyme complexes (to exploit the biotin/avidin K_d of 10^{-15}) or the use of biotin derivatives with longer linker

arms (to increase accessibility). In fact a derivative, Bio II-dUTP, with an extra seven atoms in the linker arm, is already commercially available. The day may not be far removed when you carry your Southern blot filters to lunch with you and check their progress every now and again. □

betularia reach a frequency of 80 per cent in rural East Anglia, where there is little industrial pollution⁷. None of these observations can be explained by simple directional selection by predators.

Some of the apparent anomalies in the distribution of melanic *B. betularia* are related to its powers of migration. Males migrate about 2.5 km per generation, and this results in considerable gene flow between polluted and unpolluted places⁸. Computer simulations show that a balance between selection and migration is enough to explain the persistence of typicals in highly polluted areas⁶, and partly explains why the rapid decrease in melanic frequency does not coincide with the urban boundaries of Liverpool and Manchester. However, gene flow alone cannot explain the position of this cline, nor why melanics are so common in some unpolluted parts of Britain⁹.

Mani's model incorporates new information on fitness differences between melanics and typicals which are unrelated to visual selection by predators¹⁰. Breeding experiments involving 12,569 larvae from 83 families show such that differences are quite considerable; for example, *typica* homozygotes are at an approximately 30 per cent disadvantage to *carbonaria* homozygotes and a 7 per cent disadvantage to *insularia* homozygotes when raised in the laboratory¹¹. There is no evidence of heterozygote advantage although this has often been proposed as a mechanism for maintaining melanic polymorphism. In the model, England and Wales are divided into squares with 22.5 km sides; mutation takes place at a rate of 10^{-6} per generation, and pollution in cities is assumed to have increased linearly for 55 years from 1830 and since then to have remained constant. The simulation, which incorporates experimental data on differential selection by predators, gene flow and spatially constant non-visual differences in fitness, was allowed to proceed for the 150 *B. betularia* generations from the beginning of the Industrial Revolution to the present day. It generates a distribution of melanics which is remarkably close to that found in modern populations, with an incomplete replacement of typicals even in the most polluted cities, and local patches of high melanic frequency in some unpolluted regions.

The best fit of Mani's predicted frequencies to those found in nature is obtained using estimates of non-visual

More to melanism than meets the eye

from J.S. Jones

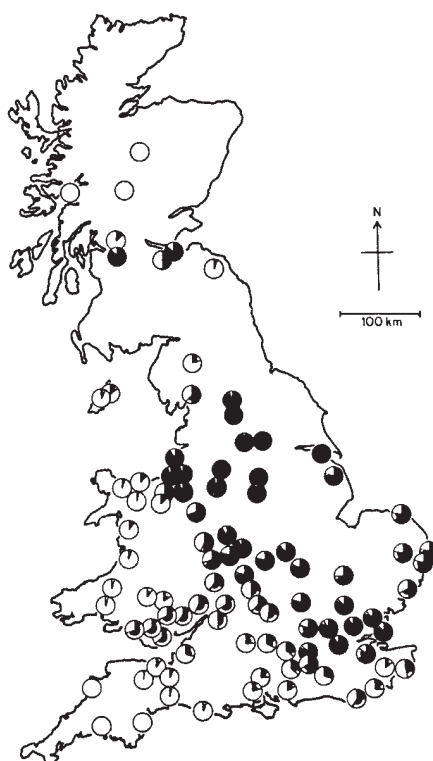
INDUSTRIAL melanism is the textbook example of natural selection in action. Like most such examples it is usually presented in a way which is both incomplete and inaccurate. The popular view is that a mutation to a black form appeared in the previously light-coloured populations of the peppered moth *Biston betularia* at the beginning of the Industrial Revolution. As in a sooty environment the melanics were well concealed from predators they replaced non-melanics in polluted cities while remaining at a low frequency in the countryside. The real story is, however, much more complex and a recent computer model, produced by G.S. Mani, a University of Manchester physicist, shows that selective predation, differences in fitness unrelated to crypsis and gene flow must all be taken into account before many puzzling features of melanism can be explained.

Melanism has always presented a number of problems. It appeared in industrial Britain not only in *Biston* and more than a hundred other moths, but in species not subject to intensive predation such as beetles, cats and birds¹. In ladybirds, melanics became more common mainly because the increased efficiency of absorption of solar energy gives them an advantage in the smoky atmosphere of cities². Melanic pigeons are favoured in towns because the associated hormonal changes make them better able to exploit the possibilities for winter breeding presented by the year-round availability of food³. In both these distantly related organisms, the mating advantage of melanic males over typicals may also be important in their evolution⁴. Although in the peppered moth the first melanic was not recorded until 1848 in Manchester, dark forms of other moths were common in forests long before the industrial revolution, and in the newly polluted cities of north-east America, industrial melanism evolved exceptionally rapidly as these variants spread from the forests. Even the apparently simple case of

industrial melanism in *B. betularia* involves at least four alleles, and many British populations contain three phenotypically distinguishable classes — the pale form *typica*, the intermediate *insularia* and the full melanic *carbonaria*^{1,5}.

There are several puzzling aspects of melanism in *Biston*. Even in the most polluted areas, such as central Manchester, the frequency of melanics never rose above 95 per cent, although field experiments on marked individuals show that melanics are considerably better camouflaged and should have replaced lighter forms long ago if only differential predation is involved⁶. When pollution was reduced the frequency of *carbonaria* fell rapidly (from 95 per cent to 82 per cent between 1960 and 1975 near Liverpool) although the melanics still appeared better camouflaged than typicals. In addition, melanic alleles in *B.*

Fig. 1 Distribution of melanics in *Biston betularia*. White sector: *typica*; black sector: *carbonaria*; halved sector: *insularia* (from ref. 1).



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1. Lees, D.R. in *Genetic Consequences of Man Made Change* (eds Bishop, J.A. & Cook, L.M.) (Academic, New York, 1981).
2. Muggleton, J. *Heredity* **40**, 269 (1978).
3. Mutton, R.K. et al. *J. Reprod. Fert. (Suppl.)* **19**, 563 (1977).
4. Majerus, M. et al. *Heredity* **49**, 37 (1982).
5. Lees, D.R. & Creed, E.R. *Heredity* **39**, 67 (1977).
6. Cook, L.M. & Mani, G.S. *Biol. J. Linn. Soc.* **13**, 179 (1980).
7. Bishop, J.A. & Cook, L.M. *Adv. Ecol. Res.* **11**, 373 (1980).
8. Bishop, J.A. *J. Anim. Ecol.* **41**, 209 (1972).
9. Mani, G.S. *Proc. R. Soc. B210*, 299 (1980).
10. Mani, G.S. *Biol. J. Linn. Soc.* **17**, 259 (1982).
11. Creed, E.R. et al. *Biol. J. Linn. Soc.* **13**, 251 (1980).

selection which differ slightly from those resulting from breeding experiments. Nevertheless, his results make it clear that the evolution of industrial melanism in *B. betularia* must depend in part on gene flow and on powerful non-visual selective forces whose nature is as yet unknown. There remain many other problems associated with this apparently straightforward example of natural selection. Why, for example, has the frequency of *carbonaria* decreased in northern England since the introduction of smoke control, but increased in the south? Even for a melanic moth it seems that life is never as simple as made out in the textbooks. □

Acetylcholine receptor cloned east and west and . . .

FURTHER to the article by C.F. Stevens (*News and Views*, 28 October 1982), it should be added that the cloning and 90 per cent of the sequence of the α -subunit of the *Torpedo* acetylcholine receptor had already been reported by a group in Britain (K. Sumikawa, M. Houghton, J. Smith, L. Bell, B. Richards and E. Barnard *Nucleic Acids Res.* 10, 5809; 1982). The group used a two-stage procedure to design a 19-base oligodeoxyribonucleotide as an efficient hybridization probe in the cloning. From cDNA sequencing, the amino acid sequence of a 24-residue pre-N-terminal signal peptide and about 90 per cent of the sequence of the α -subunit of the *Torpedo marmorata* receptor were determined. These sequences agree with the sequence for the α -subunit of *Torpedo californica* since published by Noda *et al.* in *Nature* (28 October) and reviewed by Stevens. The only differences are at positions 42 (Asn for Ser), 230 (Val for Gly) and 318 (Asn for Asp), all single-base changes which are probably due to the species difference.

The mRNA fraction used has also been shown to be that for the native receptor by an exploitation of the oocyte translation system in which it forms the functional ion channel of the acetylcholine receptor (E. Barnard, R. Miledi and K. Sumikawa, *Proc. R. Soc. B* 215, 241; 1982). □

Corrigendum

In the *News and Views* article 'A growing role for reverse transcriptase' (*Nature* 299, 204; 1982), reference was inadvertently omitted to a paper by S.Z. Hirschman, S.J. Vernace and F. Schaffner (*Lancet* 1, 1099; 1971), containing the first description of a DNA polymerase activity in preparations of human hepatitis B virus.

Pool and ridge patterns in peat mires

from Peter D. Moore

FROM the air, a peculiar pattern of concentric crescents can often be seen in the pools and ridges of a peat mire. The pattern stems from the tendency of pools on sloping mires to lengthen at right angles to the slope so that they run along the surface contours rather than downhill. But why should the peat pools grow in this manner?

Many theories have been put forward and it is possible that the causes of pool growth may differ according to the climatic environment of the mire and whether the mire surface is supplied with ground water or is dependent entirely on rainfall. Patterned peatlands are most frequent in high latitudes¹, but they occur as far south as 55°N on the western fringes of Europe to about 46°N in Minnesota², and they have recently been described as far south as 36°N in Japan³.

The boreal aapa mires occupy valleys and possess ridge and pool systems which traverse their entire width. It has been proposed that they owe their structure to ice pressure from the pool systems freezing in early winter⁴ but, although the freezing process could intensify a pattern once it has begun to form, it is doubtful whether this is the initial cause¹. Snow cover may lead to a limited development of ice in winter in many Finnish mires, yet patterns are still apparent, as they are in the patterned mires of Japan, which also receive considerable snowfall in winter.

Study of Japanese mires led Sakaguchi to put forward a different theory for the origin of the patterns⁶. He claims that the initial generation of a bank/hollow complex could result from lines of accumulation of plant detritus — 'thatch lines' — carried by flood water over the mire surface. Interestingly enough the idea was considered in relation for Finnish mires in 1920 by Auer⁴, but was not favoured by him. Sakaguchi backs up his proposals with observations on detritus patterns on sloping lawns after floods, which do indeed produce patterns which compare closely to those observed on a larger scale on sloping peatlands. It is an explanation which needs to be re-examined, preferably by detailed stratigraphical studies, in Finland.

Such a model is not satisfactory for the rain-fed raised and blanket mire pool patterns, for here the surface movement of water is restricted to local drainage requirements, there being no ground water supply. In these, the linear arrangement of pools again follow the contours of slope,

but in this case they are usually underlain by several metres of peat and the slope itself often results from the morphological features of the peat mass. The possibility that an entire body of peat may be unstable and moving gradually down slope was proposed by Troll⁷ and developed by Pearsall⁸, who considered the sloping edges of a globular peat mass on a slope as a series of concentric pressure ridges.

The idea was put to the test by Pearson⁹ at Muckle Moss in Northumberland. As a result of detailed surveying of posts within the peat body from a fixed position beyond the mire, Pearson was able to show that some posts had moved by as much as 3–6 mm over a period of seven years. The mass movement of the peat was thus confirmed and the pool patterns at this site could be explained in terms of ruptures resulting from this stress. The explanation cannot be applied to all patterned peatlands, however, for the pool morphology does not always conform to that expected of peat ruptures. Pools are often shallow and frequently they develop on very gentle slopes.

Boatman, Grade and Hulme¹¹ suggest that pools initially form in hollows which are related to irregularities in the underlying, sub-peat surface. They are then maintained by biological processes in which the peat accumulation rate is less in the vicinity of a pool than at some distance from it. The actual extent of the pools will then be related to surface water availability, one function of which is climate¹². Some recently published stratigraphical work in Caithness, Scotland by Smart¹³, shows, however, that although pool systems may have a long history, they are not necessarily persistent in the sense that they retain specific positions and expand or contract from these¹⁴. So pool patterns may change in the course of time as the mire succession and peat accretion proceeds. Fortunately, that very accumulation of peat contains a detailed record of the history of the mire and further stratigraphical studies are the obvious way to find out how the peatland patterns emerge. □

1. Sjörs, H. *Endeavour* 20, 217 (1961).
2. Heinzelman, M. *Ecol. Monogr.* 33, 4 (1963).
3. Sakaguchi Y. *Bull. Dept. Geol. Univ. Tokyo* 11, 17 (1979).
4. Auer, V. *Acta for. fenn.* 12, 1 (1920).
5. Euroala, S. *Ann. Bot. fenn.* 12, 1 (1975).
6. Sakaguchi Y. *Bull. Dept. Geol. Univ. Tokyo* 12, 35 (1980).
7. Troll, C. *Geol. Rundschau* 34, 545 (1944).
8. Pearsall, W.H. *J. Ecol.* 44, 493 (1966).
9. Pearson, M.C. *J. Ecol.* 48, 647 (1960).
10. Pearson, M.C. *Acta Univ. Ouluensis* A82, Geol. No.3, 65 (1979).
11. Boatman, D.J., Goode, D.A. & Hulme, P.D. *J. Ecol.* 69, 897 (1981).
12. Barber, K.E. *Peat Stratigraphy and Climatic Change* (Balkema, Rotterdam, 1981).
13. Smart, P.J.W. *Ecol.* 70, 549 (1982).
14. Walker, D. & Walker, P.M. *J. Ecol.* 49, 169 (1961).

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AUTUMN BOOKS SUPPLEMENT

The pitfalls of bookmaking

The making of a multi-author book can be a long and frustrating business for all those involved — contributors, academic editor and publisher. Here Anthony Watkinson charts the sequence of events and describes what can go awry.

ALL RESEARCH scientists will have written papers in journals. Quite a few who have blamelessly reached middle age will be asked to write a chapter on their specialism in a multi-author volume, that is a book of newly commissioned contributions edited by a fellow scientist. All publishers meet people who will never contribute a chapter again because the experience has been too horrific. The edited book is where the publisher-author interface is raw and bleeding. What happens, and what can go wrong?

It is likely that our neophyte contributor (let us call him Campbell) will first be approached tentatively. A letter arrives

... few publishers are actually creative.

from a well-known figure in his field, someone he met at a congress: Campbell is flattered, is likely to agree and then hears nothing more for months. Meanwhile the aspiring editor (Edwards, an energetic and ambitious man) has decided upon the field to be covered, has written a synopsis and picked a team of potential contributors, and is looking for a publisher. Contrary to general belief most books, like most journals, come to the publisher as ideas: few publishers are actually creative. Edwards's pitch (the form rarely varies) is as follows: the field is rapidly advancing/has reached a stage when a synthesis is desirable/has not been reviewed for 20 years (a chemist's argument this one); and although he would love to write a monograph, his commitments are too heavy/the literature is now too vast to be digested by an individual/the fractured nature of the field makes collaboration preferable.

There was a time when the handshake of the head of a publishing house, in the Athenaeum or over a cocktail on a roof in L.A., was a commitment enough. These days to the world outside "the publisher" is the relatively humble commissioning editor (here, Parsons). On receiving Edwards's proposal she has two tasks (the pronoun is carefully chosen on the basis of probability). First she takes advice, probably from both regular advisers and

appropriate specialists. Advisers never *like* multi-authored books; they lack cohesion etc. Nevertheless, *on balance*, the synopsis appears to cover the ground, Edwards has a good reputation and, given a few judicious additions and subtractions, the proposal is worthwhile and should be accepted.

Parsons's second task is the filling-in of forms which vary from publisher to publisher, but which all aim to establish for the ruling accountants that the book will be profitable and will be profitable quickly enough. Parsons prepares a sales forecast (by imaginative extrapolation from sales of similar books) and on the same basis proposes a price. Some publishers arrive at the price first — the market price or what libraries are thought to find tolerable — and then subtract all the costs to check that the price is acceptable. Others begin by totting up the costs and then calculate the price that will produce the profit margin required by multiplying the "unit cost" (total cost divided by print number) by a certain factor, usually about six. Subtracting from the price the percentage average discount to booksellers and agents gives net income, out of which must come production costs (obtained from typesetters', printers' and binders' scales) and the royalty payment. What remains is the gross profit, which pays all the publisher's outgoings and overheads, leaving a net profit. A second calculation based on the forecasted rate of sales establishes that there will be an acceptable return on the investment within the first year or so.

In the case of Edwards's project the way ahead is now open. The book seems to be viable both academically and commercially. Parsons's final proposal is typical: the book is to be 100,000 words (300 printed pages) in length, comprised of ten chapters, and will sell at £25 with a print run of 1,500 copies. It is time to sign contracts, in which payment for Edwards and for Campbell and his fellow contributors may be a contentious point.

Almost all academic editors are these days offered royalties but contributors not necessarily. Sometimes there is a package: the editor gets a proportion and the contributors divide the rest. Other editors get

the lot and make their own arrangements with their (probably medical) contributors. More common now is a royalty to the editor and a lump payment to the contributors, sometimes so much per page and sometimes a fixed sum per contribution, the publisher's argument being that the maintenance of tens of little royalty accounts is disproportionately expensive (overheads killing convenience like the problem of buying single nails). Whatever royalty is offered it is very likely to be a percentage of net income (list price less discount) not of list price: the computer is said to demand this. It used to be said — go for a royalty. However decreasing sales and lower real royalty rates nowadays mean that cash in hand is often preferable for the contributor.

Whatever the arrangement proposed, Campbell and his fellow contributors — maybe a little worried by now — should receive a contract within four months of first approach. If not, it could be that Edwards is hawking the book around less choosy publishers; alternatively, he has been told to shed a chapter or two and has not faced up to breaking the news to the contributors concerned.

Campbell's contract will be subsidiary to that between Edwards and the publisher. It

... it will need a mass revolt of all contributors to change the financial terms.

may be a rather scrappy piece of paper but it must *clearly* state title and length of contribution, date by which the typescript should be delivered, who holds copyright and what reward is offered. The document is likely to bristle with misspellings, not surprisingly since in many firms the job of preparation is left to the filing clerk or an inadequately briefed contracts centre. Campbell should challenge any omission or ambiguity but it will need a mass revolt of all contributors to change the financial terms.

It is usually best for all concerned if copyright for a whole book is held by one body, whether publisher or learned society.

The date of delivery must be realistic: if it is less than nine months ahead no one will treat it seriously and if it is over a year the tempo is going to be very slow. Length of contribution is often vaguely expressed: does the publisher mean printed pages or A4 double-spaced typescript, and, if printed, which format ("print area" is the relevant term here, not page size)? If given in terms of number of words, does this include space needed for figures and references? In addition offprint provision is important to some — are none to be provided, or 10, 25 or even 50? These are gratis but what about the chance of buying more? Campbell should also check on free copies: he can expect one, with the possibility of buying more at a discount.

At this same time Campbell should expect to receive more than a contract. Certainly there should be notes to authors. Because publishers continue to reprint documents drawn up by long-dead production directors in the days of letterpress, demanding imperial units, World List citations and India ink illustrations on Bristol board, such anachronisms should be challenged. Academics whose drawing offices have gone in the cuts might delicately ask about in-house help or perhaps a little advance. Wizards of the SEM should check on technical points and on how many micrographs are expected.

Even more important is guidance about the aims of the book. When Campbell was first approached he is unlikely to have received a blueprint: now Edwards must set out in particular the content, the aims and the level of writing. Are references to be comprehensive (the computer search) or selective? Campbell may be asked for a synopsis to be approved and circulated: if he is, Edwards is doing his job.

In ten months' time the postman will in theory stagger up the path of Edwards's house with ten impeccably presented type-

... even a good contributor is forced to realize that writing chapters comes after writing papers, grant applications and lectures.

scripts. In theory. Even a good editor can do little except badger the laggards with circulars and phone calls; and even a good contributor such as Campbell is forced to realize that writing chapters comes after writing papers, grant applications and lectures, and that there will be some delay: he asks for an extension of a couple of months, is given it and gets his chapter in within the year. In any one book most chapters trickle in over a period of a few months, giving the good editor time to send back for rewriting, rewrite himself, cut, argue about nomenclature, argue about facts, query the references and demand replacement illustrations — edit, in other words.

If Parsons receives the complete typescript earlier than 18 months from

contracts going out she will be lucky. There are always some dropouts and the chapter that is twice the length asked for, typed single-spaced, without margins and with illegible handwritten corrections, figures that no drawing office can handle and already screened half-tones. Campbell should be prepared for delays and demand the chance to update his chapter; he should also know that it is those projects which go on through several updatings over seven years or more that can kill friendships.

When Campbell knows that the typescript has been delivered in its entirety to the publisher (editors are quick to write to

Book publishing like house buying is a succession of delays.

say this), what sort of schedule can he then expect? Book publishing like house buying is a succession of delays: before Parsons passes the typescript to the copyeditor, until the copyeditor gets to work, while queries are sent out all over the world and answers come back, after the marked-up manuscript goes to the typesetter, and so on through the proof stage (with revised proofs if needed) and on to printing and binding. Assuming reasonable efficiency, no holding back because of cash-flow problems, a prompt postal service and no industrial action in the printing industry, reckon a year from delivery of the complete typescript to publication.

During this period decisions have at various stages to be made within the publishing house. Before money is spent on outside suppliers (typesetters, printers etc.) Parsons has to go through the same sort of procedures as she did at contract stage but this time with a real typescript. Inevitably the extent (the length of the typescript) will be greater than bargained for however tough Edwards may have been. Typesetting and printing costs may not be much higher in real terms (production controllers in publishing houses have learnt many lessons in the past few years) but the projected print run will undoubtedly be shorter because of the poor sales record of similar titles over the same period. There will usually be two calculations to be made: first on estimates, the stage which corresponds to what the contracts call "acceptance"; and second on real costs (typesetter's and printer's bills) when the final price is set. Campbell and even Edwards will know little of the in-house agonizing. Quite frequently publishers fail to remember to tell the editor the selling price of the book.

At handover of the typescript, an unworldly academic editor will hope to be given a schedule for proofs. There won't be one. If all goes well the best hope is for proofs within six months but prior warning, let alone a schedule, is most unlikely. Production staff are surprised every year by the fact that proofs going out in the summer holiday and congress season don't get returned for months. Proofs

always arrive at the most inconvenient time with an unrealistic three weeks turn-round demand, possibly reduced to a week by transatlantic postal delays. The usual system for an edited book is for contributors to return proofs to the editor, who collates them and returns a master copy to the publisher.

Suppose that the proofs are a mess. On the advice of the production department Parsons has opted for a cheap typesetter and for the money has got omissions aplenty, poor alignment of tables and an unhealthy crop of typographical errors. Campbell knows that he will be charged if the cost of making corrections is over 10 per cent of the original cost of the composition of his chapter. What can he do? He can certainly correct all mistakes that have been made by the typesetter — without fear of cost or complaint. Sometimes, however, such "mistakes" are the result of over-creative editing yet it is best to give in gracefully unless actual errors of substance have been introduced. When galley proofs were the rule authors used to rewrite in proof on the Proustian model (his galleys were famous) but the contributor of a chapter to a work of scholarship nowadays has no such chance. Each alteration means a series of actions by a highly paid operative; it can cost as much as £1 to add a comma.

Meanwhile Edwards has been contacted by the marketing department of the publisher. There is a publicity form to be filled in. The form will have asked optimistic questions about potential media coverage and the nature of the expected readership. The fact will be a badly typed regurgitation of his own summary of what the book is about, a list of places where review copies are to be sent (one hopes) and possibly requests for photocopies of the membership lists of relevant learned societies. He will not be asked about the jacket (there is unlikely to be one and the artistic design prepared by his wife may well have been lost) and there will certainly be no launching party. The best Edwards can do, poor soul, is cut down the claims (if he is a realist) and make sure that the names of all the contributors are spelled correctly.

For Campbell and his fellow contributors, the actual publication will come as an anti-climax. Edwards, being a keen editor, may have written to say that the book is "out" but his advance copy does not mean that the bookshops know about it, and it certainly does not mean that the contributors will have received their statutory free copies. Campbell, looking for reassurance, is unlikely to find it on the shelves either in the local bookshop, which received one copy on a standing order plan, or the library which bought the copy but has cataloguing delays. Publishers rarely advertise, except for a regular entry in a journal or two in which an individual title may receive much the same amount of ►

Continued on p.137

Keeping it clean

R.C. Lewontin

POPULATION genetics is the automobile mechanics of evolutionary theory. Population genetic theory is a deductive structure that asks the question: Given the mechanical details of inheritance, given mutation rates, recombination rates, migration rates, patterns of mating, the Darwinian fitnesses of various genotypes, and the relation between genotype and phenotype, how will the composition of a population or species change in time? The job of the theoretical population geneticist is to set up a mathematical machine into which the various parameters can be dumped, to turn the crank, and to produce the kinetics of evolution.

In practice this process has a number of serious difficulties. For even mildly complicated models involving several genes or non-random mating or non-constant fitness, the mathematical problems can be virtually intractable. Moreover, we do not usually know the genetic basis for traits of interest, nor are we able to measure the parameters in nature to any close accuracy.

As a consequence, evolutionists have continually searched for ways to avoid the dirty details of the kinetic equations of population genetics and to make predictions about evolution by some easier route. One form of approach has been to try to find laws of maximization or optimization, much as classical physics used Fermat's Principle of Least Action or the minimization of potential energy, in place of the kinetic equations themselves. To this class belong what R.A. Fisher called, with characteristic modesty, the Fundamental Theorem of Natural Selection, and Wright's principle of the maximization of mean fitness. Unfortunately, these maximization principles turn out not to apply generally, and the only way to know when they do is to solve the kinetic equations that we were trying to avoid in the first place.

Another attempt to make evolutionary predictions on the cheap was my introduction in 1961 of the apparatus of game theory into evolutionary biology. I had hoped that by drawing analogies — a population to the player, and the population's genetic structure and parameter

Evolution and the Theory of Games. By John Maynard Smith. Pp.218. Hbk ISBN 0-521-24673-3; pbk ISBN 0-521-28884-3. (Cambridge University Press: 1982.) Hbk £18.50, \$34.50; pbk £6.50, \$11.95.

values to a strategy — the entire apparatus of game theory could be used to make evolutionary predictions. But this attempt foundered on precisely the same rock as optimization theory: there is no guarantee that the actual kinetic process of gene frequency change will carry an evolving population to one of the so-called "solutions"

petuates a misunderstanding, for, with the exception of the metaphorical use of a few terms like "strategy" and "pay-off", Maynard Smith's theory makes no use whatever of game theoretical apparatus, but rather lies totally within the standard kinetic structure of population genetics. That is the secret of its success.

A central set of parameters for making predictions in population genetics is the set of reproductive fitnesses of the various genotypes in the population. In much of population genetic theory these fitnesses are assumed to be fixed constants characteristic of each genotype. Some progress has been made in building models that include simple dependence of fitness on population density, on the frequency of the genotype in the population, or on a fluctuating environment with known statistical properties. None of these models, however, deals with two important properties of organisms: first, any genotype may have a variety of phenotypes each with a different fitness; second, which phenotype is displayed and what its fitness is may depend upon the phenotype manifested by other individuals in the population.

This kind of doubly contingent fitness determination may make the average fitness manifested by each genotype rather difficult to compute, and so evolutionary prediction becomes very problematic. Animal behaviour in particular is full of such problems because an animal may sometimes act in one way and sometimes in another, while the fitness value of these actions will depend upon what other animals are doing. There is, then, a continuum of possible phenotypes, say from always being aggressive, through sometimes being aggressive, to always being passive, and every phenotype has a frequency-dependent fitness. And we have not even mentioned genotypes yet — not a very encouraging prospect for a theoretical population geneticist.

Maynard Smith's contribution has been to cut through this thicket by asking a somewhat more restricted question, the answer to which can be very powerful. He has developed a set of algorithms to find which of the patterns, when it is very common in the population, has a higher



in the game theoretic problem. That is, evolutionary equilibria are not necessarily the same as game-theoretic equilibria.

As luck would have it, I was visiting professor at Sussex University in 1971 when John Maynard Smith first began to reintroduce the metaphor of the game into evolutionary biology. At first, I was sceptical. The very words "Evolutionarily Stable Strategy" seemed to reek of the discredited attempts to avoid the messy realities of genetics. Only slowly did I come to understand that Maynard Smith was engaged in a totally different project, a project that is now lucidly explained in his new book *Evolution and the Theory of Games*. The name of the book unfortunately per-

fitness than any other pattern that enters the population as a rare variant. The implication is that once this phenotype is established in the population, no other can displace it. Such a "King of the Mountain" phenotype is called an Evolutionarily Stable Strategy, or ESS. It turns out that such patterns exist for a wide variety of situations in animal conflict, in learned behaviour, in resource allocation and in a variety of other very complex frequency-dependent fitness problems. The real issue is whether these patterns live up to their name. Are they really evolutionarily stable?

It is at this point that the messy details of genetics reassert themselves. If the ESS pattern can be achieved by a homozygote or if reproduction is asexual, then the answer is yes. On the other hand, if the ESS pattern can only be produced by a heterozygote, then clearly it is not achievable in pure form in a sexually reproducing population. Moreover, some populations are genetically polymorphic. Can the ESS be stably created by a mixture of genotypes obeying the rules of segregation and recombination? The answer is "Sometimes yes and sometimes no". Thus, the phenotype that Maynard Smith calls ESS might better be called the "Frequently Roughly Evolutionarily Stable Strategy". A fair amount of argument in *Evolution and the Theory of Games* is devoted to convincing us that the genetic basis of behavioural phenotypes is likely to make a FRESS into an ESS. So, for example, single locus overdominance is said, probably correctly, to be rare, and it is asserted that if many genes influence a character, it is probable that any intermediate phenotype can be produced by an appropriate mixed homozygote. It must be pointed out, however, that epistatic gene interaction may be very important and that linked genes will produce a pseudo-overdominance that decays only slowly, if at all.

Other criticisms have been made of ESS theory, but Maynard Smith discounts them as being beside the point. So, predictions of ESS depend upon the set of alternative "strategies" that are evaluated. But precisely the same is true of all kinetic predictions in population genetics, which are based entirely on the alternative genotypes actually present in the population. Indeed, ESS theory has the advantage over vague optimization theories that it makes very explicit what the set of alternatives are that are being considered. ESS theory is not easy to check against natural observations because both the parameters and the predictions are usually in the form of relative frequencies that have immense standard errors, unless sample sizes are very large. But, again, that is a problem for all of population genetics, so ESS predictions are no more unfalsifiable than anything else in evolutionary genetics. The criticisms are to the point, however, when ESS theory is

used uncritically, and it must be admitted that the very words "Evolutionarily Stable Strategy" have a kind of puffery about them that invites the same sort of over-generalization as the not-so-Fundamental Theorem of Natural Selection. After all, evolution has been going on for three billion years, and if some strategy is evolutionarily stable, why surely that must mean that it is here to stay. The letters ESS now rival DNA as the most fashionable acronym in biology, and we may expect that many vulgarizers both outside and within evolutionary biology will simply not appreciate the contingency of the method.

There is a deeper sense in which ESS theory shares a flaw with all attempts to make general explanations for evolutionary events. At any moment we will see characters that are common to all members of a species, and we will be tempted to ask "Why is the species like that?". To give the answer "Because that is its optimal phenotype" is to give a totally uncheckable answer because we cannot ever know what set of alternatives was available to the species during the course of its evolution to

its present state. To say that the codfish lays millions of eggs in order to maximize the number of survivors is to forget that it *might* have become ovoviviparous and so have provided maternal care. The same problem exists for ESS theory. We simply cannot say that a species is at an ESS because we do not know what set of alternatives was available. That is, such theories have predictive power but not explanatory power, *post hoc*.

On balance, ESS theory, because it is so firmly rooted in the kinetics of population genetics, must be judged as one of the more fruitful directions in theoretical evolution. Like other attempts at making generalized rules, such as the Fundamental Theorem of Natural Selection, or the principle of maximization of mean fitness, it works when the details of the genetic system are appropriate, but not always. Thus, we are still not free to ignore the material particularities that underlie phenomena. When all is said and done, "God is in the details". □

R. C. Lewontin is a Professor in the Museum of Comparative Zoology, Harvard University.

The man who was crunched by numbers

Richard L. Gregory

Charles Babbage: Pioneer of the Modern Computer. By Anthony Hyman. Pp.350. ISBN 0-19-858170-X. (Oxford University Press: 1982.) £12.50.

ANTHONY HYMAN'S biography of the inventor of programmable computers is no dry account of a premature technological innovation; and neither is it, as a previous biography, a shallow journalistic treatment of this truly remarkable man. Hyman has succeeded in bringing Babbage alive in the social context in which he played, as we may now appreciate, such a prominent part, and gives a clear account in just sufficient detail of Babbage's outstanding achievement, his Analytical Engine.

Charles Babbage (1791-1871) was born of a West Country banking family and became Lucasian Professor of Mathematics at the University of Cambridge (1823-1839). Elected to the Royal Society in 1816, he played a prominent if abrasive part in the scientific and technological life of his time; his influence extended to continental Europe, especially to Italy. As is well known, with advancing years he became a bitter and somewhat disappointed man; but this book shows the extraordinary range of his enthusiasms and his love of life. In retrospect he is far more to be envied than pitied, in spite of his famous defeats at the hands of Government, especially Peel's in the 1840s. Perhaps surprisingly, 20 years earlier Babbage was imaginatively supported by

the Duke of Wellington when he was Prime Minister. As Hyman says (p.191):

The underlying cause for this sad change was the developing Victorian academism. A wide gap was opening between the superior pure science of the Universities and inferior applied science, the bread and butter of the engineers. Babbage's engines fell through this gap into a century's oblivion.

Those engines were only understood by a few people, among them Byron's daughter Ada, Lady Lovelace. Most difficult to grasp was the notion that in its progress towards solutions such a machine could be affected by its own findings. Wheeled calculators were known from Pascal's machine of 1642, so concepts of representing numbers and carrying out arithmetical and logical operations were fairly familiar — and indeed have been from the prehistoric abacus — though these vitally important concepts have received remarkably little comment or appreciation from philosophers; or perhaps even more surprisingly, from mathematicians or astronomers, or financial people who might have benefited greatly.

Like Helmholtz's Unconscious Inference and Freud's Unconscious Wishes, perhaps Babbage's computing engines were an unwelcome threat to human dignity; and to social and moral responsibility, for consciousness then as it still does, seemed necessary for blame and praise. Babbage's own attitude to blame

and praise is convoluted. He rejected a knighthood, while also confessing to Ada that accolade and honours were particularly dear to him. He was justifiably upset that his Analytical Engine was excluded from the Great Exhibition of 1851; though he was no doubt delighted that his friend Charles Dickens based the HOW NOT TO DO IT OFFICE of *Little Dorrit* on Babbage's experience of Government's attitudes to those who have the wit to do new things.

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Charles Babbage, a man with a vision

Such contrasts run right through Babbage's life. He was so active in the society of the time that through Hyman's account one learns a great deal about this formative period of science and its relations with technology, as well as about Governments and how they dealt with novelty. Babbage was a man with a vision of the future industrial world as well as of computing; he was influential in the founding of several learned societies, as well as in the application of pure science in industry and commerce. He had an extremely active social life, too, being friendly with the great men of his time, and

also appreciated the arts; on a splendid occasion when he was bored by a performance of Don Giovanni, he went behind the scenes and virtually invented modern theatre lighting. He invented the ophthalmoscope, and indeed everything he turned his hand to was affected by his touch. Babbage not only saw how the punch card system of Jacquard's loom could be used to control logical processes in a machine — and so how thought itself can be carried out by machinery — but, by almost superhuman effort, he developed precision engineering and mechanical drawing to a point where the general-purpose computer became very nearly if not quite a practical possibility. The full realization required the technology of electronics, but the basic concepts are there in beautifully fashioned cog wheels. (One is almost tempted to say cognitive wheels!). Babbage did as much as any man to create the machinery and the techniques — including engineering drawing — for precision mass production which has produced historically unimaginable wealth and knowledge.

All of this was achieved at the cost to himself not only of a large proportion of his personal fortune, but also in having to live with a painful gap between what he could see was needed and possible, and — for him — the stupidity and inertia of the society in which he lived. It is just this tension between his dream and our present reality that places Babbage as a man uniquely poised at the threshold of our age — leading surely to fully intelligent machines.

Anthony Hyman has succeeded in bringing the many themes of Babbage's experience and contributions together. His book is eclectic and thoughtful, as well as being both an inspiration and a warning to those of us who have faith in invention rather than received wisdom. □

Richard L. Gregory is Professor of Neuropsychology and Director of the Brain and Perception Laboratory at the University of Bristol.

Mary Evans

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Babbage's Analytical Engine, which stands in the Science Museum in London. The machine was never completed. Babbage had thought out all of the principles on which modern computers function, but not until a century later, and the rise of electronics, could his ideas be fully realized.

Entombed in print

P.B. Medawar

A Biographical Dictionary of Scientists, 3rd Edn. Edited by Trevor I. Williams. Pp.674. ISBN 0-7136-2228-8. (Adam & Charles Black: 1982.) £15, \$30.

THIS admirable book first appeared about 12 years ago and the call for a second and now for a third edition is evidence enough of its fulfilling a real need, for although it is good value it is not cheap. The innovations of the present edition are the addition of supplementary biographies of some 40 scientists who have qualified for admission — as one day we all shall — by dying, for all is in the past tense. Another valuable innovation is the inclusion of an index which under a variety of subject headings lists the names of the scientists specially connected with each one. Thus under the subject heading "Evolution" we are referred to von Baer, Darwin C, Darwin E, Dobzhansky, Haeckel, Hooker, Huxley, Lamarck, Malthus, Maupertuis, Nägeli, Naudin, Pringsheim N, Spencer, Vallisneri and Wallace — which is fair enough.

It was a claim of the first edition that it addressed general readers as well as the student of science but it really does not go very far to appease the former: the entries are baldly factual.

In this edition the grounds for inclusion of a name in the main body of the text remain as mysterious as ever (the task of deciding must have been extremely difficult). The omission of such a prominent figure as Darcy Wentworth Thompson can I suppose be excused on the grounds that he did not found a school and is not now thought of as a man who was scientifically very influential, though he was greatly admired. Clearly priority has always gone to practising scientists rather than to men who are not primarily known as or indeed known to be scientists, however great their influence on the general public, on fellow scientists or on literature or the arts.

Thus there is no place for Goethe though he did some work which he probably thought of as scientific (the "vertebral theory of the skull", for example) and his commitment to *Naturphilosophie* which exercised a wide influence — undoubtedly a harmful one. But I was sorry not to see H.G. Wells who, although he held only a fairly minor teaching post in zoology, was the author of a widely consulted zoological text and had a profound influence on the young through his brilliantly imaginative short stories which were greatly superior in all respects to those of Jules Verne. Francis Bacon rightly rates inclusion, however, because of his profound influence in throwing off the hegemony of Aristotelian thinking and propounding a philosophy of science which is recognizably congruent with modern thought even if it was in some ways pretty wrong headed. He was not

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Edward Arnold

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much of a scientist though, and one can understand why one of his biographers called him "a medieval philosopher haunted by a modern dream".

I did not spot any errors of fact but then, I was not looking for them, taking it quite for granted that a work of such great compass and of so many hands must surely contain a few mistakes, and being unwilling to incur the deserved odium of

picking them out and calling attention to them. In the outcome I rate this dictionary as a great success and a considerable feat of editorship and of publishing which will earn the continuing gratitude of all who have occasion to refer to it. □

Sir Peter Medawar is a member of the Scientific Staff of the Medical Research Council's Clinical Research Centre, Harrow, Middlesex.

Along the lines of Einstein's thought

Arthur I. Miller

'Subtle is the Lord . . .': The Science and the Life of Albert Einstein. By Abraham Pais. Pp.552. ISBN 0-19-853907-X. (Oxford University Press: 1982.) £15, \$25.

"SUBTLE is the Lord, but he is not malicious", remarked Einstein to a colleague one day in 1921. When queried further on this statement he replied that "Nature hides her secret because of her essential loftiness, but not by means of ruse". Einstein's life-long passion was to pluck from the cosmos the laws of nature ("her secret") that exist independently of the scientist as knower. And what a passion it was.

During the period from 1905 to 1916 Einstein set the foundations of twentieth century physics with two lines of research. One line dealt with invariance principles and culminated in the special (1905) and general (1915) theories of relativity. Then there were the statistical researches that resulted in a theory of Brownian motion (1905) and a route to the verification of atomic structure, the light quantum hypothesis (1905), the first quantum theory of the solid state (1907), the wave-particle duality for light (1909), and the A and B coefficients (1916) that would lead to the laser. Finally, in 1925 came one more major piece of creative work, namely his argument, independent of Louis de Broglie, for the wave-particle duality of matter.

Thematically Abraham Pais's "scientific biography" of Einstein is set out along those two lines of Einstein's research because "better than anyone before or after him he knew how to invent invariance principles, and make use of statistical fluctuations". After 1916 these two strands merged into Einstein's quest for the unified field theory, an episode in the history of science that Pais explores systematically for the first time.

Among Pais's informative biographical sections are those describing how Einstein's feats during the intensely creative period of 1905-1916 were accomplished in the face of events that would have distracted all but the most determined: he worked dutifully eight hours per

day at the Patent Office in Berne (1902-1909); he moved between academic positions at the University of Zurich (1909-1911), the German University in Prague (1911-1912), back to his alma mater that had virtually disowned him in 1900, the ETH, Zurich (1912-1914), and then to the summit of the scientific world, the University of Berlin and the Kaiser Wilhelm Institute (1914-1933); he married Miliva Maric in 1903, by whom he had two sons before they separated in 1914; and he had to deal with health problems and the deprivations of war time. Little wonder that in December 1915 after completing the theory of general relativity Einstein wrote to the venerable H.A. Lorentz that he was "satisfied but rather worn out [*kaputt*]".

The episodes written with the most verve are those describing Einstein's tortuous path to the general theory of relativity and then his work towards the unified field theory. The combination of new material and infectious prose in these chapters conveys to the reader some of the excitement of doing science.

As an example consider Pais's description of Einstein's work towards the general theory of relativity. In 1913, owing to Einstein's hitherto unsuccessful research towards generalizing special relativity, Pais describes Einstein as one "who stands alone . . . possessing only a vision" of which he is "supremely confident". Let us fill in a bit. Since 1907 Einstein had been struggling to generalize special relativity to include accelerating reference systems and gravity. A key realization, Pais writes, was that he had to "start all over again". Pais argues cogently that while in Prague Einstein had done just that by using a curved space-time that required a mathematics with which he was not conversant. "Grossmann, you must help me or else I'll go crazy", he said to Marcel Grossmann his former classmate at the ETH upon rejoining him there as a colleague in 1912. There followed a joint paper with Grossmann who wrote the mathematical portion, polemics with the "fearsome" Max Abraham, and exchanges with Gustav Mie and Gunnar Nordström. These men offered alternative gravitational theories

couched in variations of a four-dimensional flat space-time the "belief in which amounts, I feel, to something like a superstition", wrote Einstein to Erwin Freundlich in early 1914. Pais concludes that "Only Lorentz had given him some encouragement". In 1913 the doyen of German physics, Max Planck, listened to Einstein describe his work towards a general relativity theory and then told him that even if he succeeded "no one will believe you".

The onset of war does not affect Einstein's energy; the war years "rank among the most productive of his career". Amidst the tumult about him and his unsuccessful research on gravitation, in early 1915 Einstein turned to experiment in work on magnetism with Wander Johannes de Haas.

Then, in November 1915, the breakthrough occurred for the gravitation problem. He returned to the requirement of the general covariance of the field equations that he had "abandoned only with a heavy heart" in 1912. This time Einstein knew he was on the right track, because, Pais writes, "one week before the general theory of relativity was completed [25 November 1915] Einstein obtained" the correct result for the advance of Mercury's perihelion. Pais continues:

This discovery was, I believe, by far the strongest emotional experience in Einstein's scientific life, perhaps in all his life. Nature had spoken to him. He had to be right.

There followed Einstein's prediction for the bending of light near the Sun and its verification by Arthur Stanley Eddington's Solar eclipse expedition in 1919; then was "Einstein canonized".

After 1920 Einstein left the mainstream of scientific research to pursue his vision of a generalization of relativity that unified electromagnetism and gravitation. The generalization would be based on the themes of continuity and classical causality, themes that were antithetical to the emerging atomic physics of Niels Bohr, Max Born and Werner Heisenberg. Pais asserts that Einstein had always considered a quantum theory as "provisional". He traces this strand in Einstein's thinking in great detail starting from the 1905 light quantum paper, complete with an illuminating analysis of the Bohr-Einstein dialogue of 1927-1930. Unfortunately, Pais rejects abruptly the important philosophical-physical analysis in the Einstein-Rosen-Podolsky paper of 1935 by quoting one of Bohr's *et cathedra* pronouncements of complementarity. Pais's reason, as he states straightaway in

his book, is that "I would say that at his best [Einstein] was not" a philosopher.

Pais realizes that Einstein's decision to pursue a unified field theory poses a profound question that is ripe for historical analysis:

Why does this man, who contributed so incomparably much to the creation of modern physics, remain so attached to the nineteenth century view of causality?

In outline his reply is: Einstein's infatuation with unification is evident from the beginning, as seen in his reaction to momentarily promising results from a 1901 paper where he compared intermolecular and gravitational forces — "a wonderful feeling to recognize the unifying features of a complex of phenomena", wrote Einstein to Grossmann in 1901. Although Einstein quickly abandoned the 1901 results, Pais considers that "this wonderful feeling sustained him through

life". Then there was success with unification in special relativity (electricity and magnetism) and general relativity (geometry and gravity). These results, continues Pais, led him on a "quest for harmony" (geometrization of gravity and electromagnetism) based on the continuity and classical causality inherent in the differential equations of a classical field theory.

Here, and at other key points, it would have been appropriate for Pais to have developed Einstein's philosophical analysis of physical theory. Analyses of Einstein's scientific research that take seriously contemporaneous philosophical currents reveal that he was indeed a philosopher-scientist. While some lines of his eclectic philosophical view remained constant, others were transformed by his scientific research. Some investigation into this dimension of Einstein, and into other

issues that are not readily apparent from the primary scientific literature, would have lent more depth to Pais's reply to a key question in the history of ideas. Pais's choice to exclude philosophical issues and his sparse use of recent historical scholarship is most costly to his analysis of special relativity theory — for example, on Einstein's invention of the theory and the theory's interpretation by Lorentz, Planck and Henri Poincaré.

Pais's forte is in the domain that he set out for himself; namely, to blend expository accounts of Einstein's complex physics with his scientific correspondence. This is no mean feat. A particularly valuable part of the book is where Pais goes on to bring research on gravitation and unification, as currently interpreted, up to date.

Abraham Pais is a distinguished physicist in his own right; his scientific biography of Einstein has clearly been a labour of love. The level of the scientific presentation is high, but so are the rewards, and the book fills in one part of the mosaic that is Einstein, his life, his times and his science. Together with such general biographical works as P. Frank's *Einstein: His Life and Times* and B. Hoffmann's *Albert Einstein: Creator and Rebel*, Pais's examination of Einstein's scientific oeuvre will be an important acquisition for both physicists and historians and philosophers of science. □

Arthur I. Miller is University Professor of Philosophy and History at the University of Lowell, and Associate of the Physics Department at Harvard University. He is author of *Albert Einstein's Special Theory of Relativity: Emergence (1905) and Early Interpretation (1905-1911)* (Addison-Wesley, 1981).



Einstein in March 1955, a month before his death.

From 'Subtle is the Lord'...

Myths of religion, myths of science

John Habgood

Abusing Science: The Case Against Creationism. By Philip Kitcher. Pp.213. ISBN 0-262-11085-7. (MIT Press: 1982.) \$15, £10.50.

CREATIONISM, or "creation science", claims to provide a scientific alternative to orthodox evolutionary theory, in terms which bear a suspicious resemblance to a literal interpretation of the first chapters of Genesis. Its proponents are capable of stating their case with a display of scholarship which looks convincing to those who are not experts in biology, and their plea for tolerance, for a "balanced treatment" of creationism and evolution in school curricula, has made some disturbing headway in the United States. In alliance with the Moral Majority, creationists are now seen as a formidable threat by educationists, scientists and non-fundamentalist theologians. As Philip Kitcher puts it "... although the Creationist campaign is advertised as an assault on evolutionary theory, it really constitutes an attack on the whole of science".

Kitcher's book is an impressive and absorbing counter-attack, which subjects all the main creationist arguments to detailed criticism. Those arguments are many and various, ranging from the

accusation that evolutionary theory is unfalsifiable, to determined efforts to falsify it by appealing to palaeontology, thermodynamics, statistics, geology, uncertainties about transitional forms in the evolution of species — in fact any argument, however ancient or discredited, which seems to throw doubt on the evolutionist case. Kitcher relentlessly exposes the confusions, misunderstandings and misrepresentations on which the creationist platform is based, and provides, as he says, "a manual of intellectual self-defense" for those who find themselves under pressure. It is a splendid piece of demolition, but the effect is not merely negative. A valuable spin-off is his clear demonstration of the difference between science and pseudo-science, and this is why the book should be of interest even to those who stand outside the immediate controversies.

Why do intelligent people indulge in such silliness as creationism? The motive, despite tactical disclaimers to the contrary, is plainly a religious one, and it is a sad fact that for many Christians, particularly in the United States, Biblical literalism is still the rock on which faith is built. Kitcher attempts to meet this problem in a final chapter by a sympathetic treatment of religious faith, but the account is sketchy and this is the weakest part of the book.

The fact that creationists link their attacks on evolution with general denunciation of atheism, communism, nazism, behaviourism, racism and every other bugbear of liberal and illiberal thinking, is an extreme expression of a much more widely felt malaise. The fear that science can be dehumanizing is not confined to cranks. Sensitive scientists have always known that they are engaged in a human and fallible activity which entails adherence to some basic human values. But popularly, and in the mouths of some of its exponents, it does not come across like that. A hard, debunking kind of science which cuts human beings and their aspirations down to size too brutally, provokes its own reaction. The cure for creation science and its like, therefore, lies deeper than the arguments mustered here, important though they are. Scientists may claim that it is not their business to take care of human self-esteem, and that in any case the theory of evolution, by showing us where we have come from, does not reduce our worth. This is fair comment. But it is also true that the refusal to face questions about human worth in the name of scientific objectivity can easily give the impression that such questions do not matter.

It is not enough, therefore, to expose religious obfuscation. The appeal of movements such as creationism will not be undermined until ordinary people feel in their bones that religious and scientific experience can be properly integrated.

Abusing Science is a useful first step in clearing away some of the rubbish.

I have, however, one small niggle about the book. Kitcher begins by referring to the famous encounter between Bishop Wilberforce and T.H. Huxley at the British Association in 1860. This has become part of the mythology of science, but it is a gross libel on Wilberforce to classify him as a nineteenth-century equivalent of today's creationists. In his day there were good scientific arguments to be levelled against Darwinism, and the fact that he used ridicule unwisely does not affect the genuineness of the issues at stake. The point about creationism is that the arguments are now known to be worthless, so that twentieth-century religious polemic in pseudo-scientific dress belongs to quite a different moral dimension. Scientific myths, like religious ones, sometimes need to be allowed to die. □

John Habgood, formerly a Demonstrator in Physiology at the University of Cambridge, is Bishop of Durham.

Curing nonsense

Stuart Sutherland

Pluto's Republic. By Peter Medawar. Pp.351. ISBN 0-19-217726-5. (Oxford University Press: 1982.) £12.50, \$25.

THE intelligensia's attitude to science is becoming increasingly ambivalent. While resenting their dependence on some of its products and fearing the dangers of others, they rejoice at the least hint that there may be something wrong with a well-established scientific theory. This propensity is encouraged by the wilful ignorance of newspaper correspondents, as witness the recent nonsensical attempt to treat cladistics and punctuation as disproofs of Darwinian theory.

Yet despite this aversion to science, nothing is more calculated to lend credence to shoddy thinking than the claim, however far fetched, that it is scientific. Psychoanalysts repeatedly make this assertion about their subject and many universities to their shame have attempted to conceal the vacuity of their social studies courses by calling them "social sciences". In a recent tome on English literature, Mr Leon Edel even claims to be practising "scientific" literary criticism. It is a pity that those in arts subjects have so little faith in what they are doing that they feel obliged to dignify it with the name of science. The confusion of the intelligensia is further illustrated by the fact that they increasingly take refuge in the gibberish of such authors as Lacan, Laing and Levi-Strauss and in such creeds as ethnomethodology, where nothing is true but thinking makes it so.

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Unfortunately, few scientists have the time or the patience to expose the nonsense produced by what Sir Peter Medawar calls "the intellectual underworld", and we should be grateful to him for appointing himself a defender of reason and for untoppling some of its assailants. It is true that most of the denizens of *Pluto's Republic* are more fools than knaves, but they are none the less harmful for that.

The book incorporates two of Sir Peter's previous collections of essays, and also includes a dozen or so essays not previously published in book form. It is pleasant to re-read such classic exercises in debunking as his reviews of Teilhard de Chardin's *The Phenomenon of Man* and Arthur Koestler's *The Act of Creation*, even though both books are now largely forgotten. Nothing is more difficult than exposing nonsense since one cannot refute an argument where none exists. For the most part Sir Peter exposes the authors he is attacking by direct quotation, though he cannot resist adding his own ironical asides. For example, having quoted from Teilhard "Consciousness was now leaping up and boiling in a space of super-sensory relationships and representations", he adds "The analogy . . . is with the vaporisation of water when it is brought to boiling point, and the image of hot vapour remains when all else is forgotten". He is also good at showing how a thesis leads to untenable conclusions. He points out, for instance, that since according to Koestler laughter is produced by the "bisociation" of two incompatible "matrices" of thought, the disproof of a scientific theory should produce gales of laughter. As Sir Peter remarks, "We are not amused". Irony fails him in the face of the fatuity of a passage from one of R.D. Laing's associates, David Cooper: "Curing usually implies the chemical treatment of raw materials so that they may taste better, be more useful, or last longer. Curing is essentially a medical perversion". Sir Peter's recurrent assaults on psychoanalysis are, however, more amusing than original.

His onslaughts are interspersed with the Johnsonian thump of common sense. He remarks, for example, of dreams that they may merely be noise conveying no information whatsoever, and of Levi-Strauss's insistence on the scientific validity of the Siberian myth that the touch of a woodpecker's beak will cure tooth ache: "Would not someone actually suffering from tooth ache incline towards a more pragmatic style of belief?". His common sense is also illustrated in a recent essay demonstrating that economists can never make accurate predictions: among other obstacles is the fact that the predictions may themselves influence the state of the economy. And he presents a sensible defence of genetic counselling: "Scientific evidence bears on decisions which are not of course themselves scientific".

He also resembles Dr Johnson in his capacity to write with an air of authority

even — as occasionally happens — on topics he seems to know little about. For example, he asserts that intelligence cannot be measured by a single number and that there cannot be such a thing as a "culture free" test of intelligence, but he makes no reference to the extensive research on both topics.

About a third of his new book is devoted to the philosophy of science. Although these essays are a little repetitious, Sir Peter provides a clearly stated summary of Karl Popper's position, to which he has added with considerable historical erudition an account of the work of some of those, such as Whewell, who anticipated Popper many years ago. One cannot help wishing that Sir Peter had taken up his sword to destroy some of Popper's apostates such as Feyerabend, whose confusions are as much in need of exposing as those of Teilhard or Koestler.

Although not everything Sir Peter has to say is original, it is always well said. It is a truism that among academics scientists write better than those in arts subjects, but he writes exceptionally well even for a scientist. He is never short of an apt quotation, a nicely chosen simile ("They accepted the contemporary and far from adequate form of Darwinism in much the same way that nicely brought up people accept their religion, that is, in a manner that contrives to be both tenacious and perfunctory"), or a witticism ("Haldane was totally lacking in worldly sense, a sulky

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Sir Peter Medawar — the Johnsonian thump of common sense.

innocent, a whole-hearted believer in *Them* — the agents of that hidden conspiracy against ordinary decent people, the authorities who withheld the grants he had never asked for and who broke the promises they had never made").

Sir Peter has invented a literary genre of his own, which might be called *bellesciences*, though he is perhaps at his best when he is exposing nonsense, of which there is still an abundance. If he thought *The Act of Creation* boring, let him try reading Lacan. □

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Nuts about PK

Martin Gardner

Psychokinesis: A Study of Paranormal Forces Through the Ages. By John L. Randall. Pp.256. ISBN 0-285-62540-3. (Souvenir Press: 1982.) £8.95.

MANY worthless books by writers who call themselves parapsychologists have been published in recent years, but *Psychokinesis* by John Randall, a biology teacher at Coventry School, tops them all. It is not just that he rakes over stale ground but that his book is hopelessly out of date.

Consider, for example, his enthusiastic endorsement of the psychokinetic (PK) powers of Uri Geller, the Israeli magician turned flim-flam artist. Randall makes much of John Taylor's high praise of Geller, and of the spoon-bending children featured in Taylor's book *Superminds* (Macmillan, 1973). He never informs his readers that in 1978 Taylor executed a 180-degree turn and denounced all psychic metal bending as fraud. Taylor even wrote a book about his disenchantment: *Science and the Supernatural*, published by Dutton in 1980.

Consider the pages in which Randall rhapsodizes over Geller's alleged alteration of the chemical structure of a piece of nitinol wire, giving it a new "memory" which experts could not remove. That is totally false. Eldon Byrd was in error when he made this sensational claim, reporting on his nitinol tests with Geller in Charles Panati's now discredited anthology, *The Geller Papers* (Houghton Mifflin, 1976). Experts at the Lawrence Livermore Laboratory, in California, removed the wire's memory easily. This was carefully detailed in my paper, "Geller, Gulls and Nitinol" (1977) (reprinted in *Science: Good, Bad and Bogus*), but either Randall never read it or, what is worse, read it but did not want to mention it.

Consider too the section in which Randall extolls the "thoughtography" of Ted Serios, a Chicago bellhop, who for a short time was apparently able to project onto Polaroid film his memory of photographs he had seen earlier in magazines. No one, says Randall, ever found evidence of trickery. Nonsense. In 1967 Charles Reynolds and David Eisendrath published in *Popular Photography* a complete exposé of how Serios performed his whimsical trick. Ted has been unable to replicate it since, although magician James Randi demonstrates it regularly and with more skill. Did Randall know of this exposé? If not, his research was amateurish. If he did, his failure to discuss it is reprehensible.

The sad fact is that Randall buys almost everything on the psi scene no matter how flimsy the evidence. He believes the PK is behind the spirit rappings and the table liftings produced by great mediums of the

past. Many pages describe the levitations of human bodies from early Christian saints to meditating yogis; Randall suspects that PK helped Nijinsky make his high leaps on the ballet stage! He is persuaded that PK can hurl heavy objects across a room, move the hands of a clock and cause light objects, such as matches and pill boxes, to "walk" across a table.

A postscript summarizes the 1981 "minilab" phenomena recently disclosed by W.E. Cox, a former associate of J.B. Rhine and a confirmed Gellerite. Leather rings link and unlink. A pencil rises in the air and writes on a notepad. Randall assures us that Cox captured all these wonders on film, but he doesn't reveal that the films are peppered with time breaks, and that Cox's efforts to prevent fraud were so minimal that when he presented his stop-action films at a parapsychology conclave last year in Syracuse, New York, they were greeted with derision.

Randall even thinks PK is behind an old parlour trick in which four people use extended little fingers to hoist a heavy person from a chair. Samuel Pepys describes it in his *Diary* as a pastime of French schoolgirls. Geller does this stunt often in his stage act, using a fat man from the audience and three volunteers to assist him in the lifting. (By including himself among the lifters Geller can make sure the lift fails on the first attempt when PK forces are not invoked.) Hilarious photographs of Colin Wilson being "levitated" in this manner are in his preposterous book, *The Geller Phenomenon* (Aldus Books, 1976). "As far as I know", writes Randall, "no physicist has ever given a 'normal' explanation of this phenomenon".

What is there to explain? The first lift is uncoordinated. When it is repeated, after a preliminary ritual of some sort, the four lifts are synchronized, the weight is thereby evenly distributed four ways, everybody tries harder and up goes the fat man.

Randall believes everything. Well, not quite. He is surprisingly sceptical about what parapsychologists call "animal psi". When Helmut Schmidt's cockroaches seemed to use their PK to bias an electronic randomizer, Randall suspects it was not cockroach PK that did it, but PK from Schmidt or one of his assistants. Randall himself has published papers on how an experimenter's PK can influence animal life; in one case it modified the paths taken by crawling wood lice, in another test, the direction in which gerbils jumped. He was unable to replicate the positive results of a 1952 experiment by Nigel Richmond in which Richmond's PK influenced the paths of swimming paramecia.

To explain PK Randall dredges up an old theory, popular with Spiritualists a hundred years ago, that psychics somehow have the power to shift matter in and out of hyperspace. This was the opinion of a German physicist Johann Zöllner, to whom Randall devotes an entire chapter.

Zöllner used the theory to account for the sensational results of his experiments with the famous American slate-writing medium, Henry Slade. His book about Slade, *Transcendental Physics* (English translation, 1880), is almost as funny as Jule Eisenbud's *The World of Ted Serios* (Morrow, 1967). Randall thinks I was unfair to Zöllner when I once called him "a remarkably stupid fellow". On the contrary, Randall is convinced that Zöllner was an astute investigator of Slade's genuine powers.

Randall's bibliography of 193 references is notable only for its omissions. Where, for example, is Edward Girden's classic paper, "A Review of Psychokinesis" (*Psychological Bulletin* 59, 353-388; 1962)? In view of Randall's claim that magicians have no idea how Geller does his tiresome tricks, why does he neglect to list two books on sale in magic shops, *Confessions of a Psychic* (Karl Fulves,

1975) and *Further Confessions of a Psychic* (Karl Fulves, 1980), both by Uriah Fuller, which give Geller's techniques in full detail?

Uninformed readers may find Randall's book persuasive, but most professional parapsychologists will be as embarrassed by it as they are by the scribbles of such irresponsible journalists of the occult as Colin Wilson, Lyall Watson and D. Scott Rogo. *Psychokinesis* is only the latest, but surely not the last, of a seemingly endless line of lurid books about the paranormal, hacked out by gullible believers who are incapable of distinguishing competent investigations from shabby research and anecdotal poppycock. □

Martin Gardner is a science writer, formerly with Scientific American and now a regular contributor to Discover. His most recent books are Science: Good, Bad and Bogus (Prometheus, 1981) and Aha, Gotcha! (W.H. Freeman, 1982).

Neotony: is the child father to the man?

P.E. Bryant

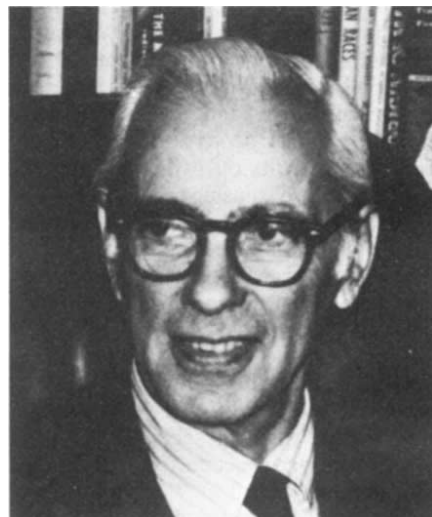
Growing Young. By Ashley Montagu. Pp.306. ISBN 0-07-042841-7. (McGraw-Hill: 1981.) \$12.95.

It is easy to detect a certain general ambivalence about the significance of childhood. On the one hand children seem to have some admirable and quite distinctive talents: they learn, for example, some very complicated things such as language (and sometimes two or more languages) with astonishing ease and they impress us with their quickness and their inventiveness. But on the other hand child psychologists keep on telling us that children lack many essential and therefore desirable qualities, and indeed these professionals devote much energy and ingenuity to the goal of hurrying on children's "development" and making them more like adults as quickly as possible.

Several people seem to manage to take both points of view at the same time even though the two obviously contradict one another. Ashley Montagu is not one of these people. His book is devoted unambiguously and enthusiastically to the proposition that childish qualities are not only desirable: they are, he thinks, precisely what distinguishes us from other species.

There are two parts to his thesis, the first anatomical and the second behavioural. The first part is the more familiar of the two and it also has more empirical support than the second. Neotony, a theory originally put forward by Bolk and lately extended in a well-known book by Stephen Gould (*Ontogeny and Phylogeny*; Harvard University Press, 1977), is Ashley

Montagu's starting point. He takes up the argument that adult human beings preserve more of the physical features which characterize their childhood than do the adults of other species. Moreover, he argues, childhood takes up a larger proportion of the life span of humans than it does of other species. This, as far as the author is concerned, is not just a quantitative difference. He dwells on the evidence that human babies are born at an early



Ashley Montagu — enthusiasm for neotony.

gestational stage, and suggests that this is a significant factor in the quality of human social relationships. His argument is that much of family life is influenced by the need to protect and nurture helpless human infants.

Having made this case persuasively and clearly the author then tries as well to make a direct connection with behaviour, and

here he is notably less successful. His theory is that neotony is not just a matter of anatomy; human behaviour too, he claims, is strikingly "neotonous". The author attributes man's evolutionary success to the retention of traits which are characteristic of human childhood. These so-called "neotonous traits" include curiosity, creativity and sensitivity. Ashley Montagu's idea is that these are childish things which adults, and particularly those who are successful or at least admirable, retain through their lives.

There are several things wrong with this second part of the book. One is simply that the author rarely produces anything like convincing evidence for his thesis. There never has been any evidence at all that children are more creative than adults, for the very good reason that we have never had any good measures of creativity. The author's discussion of what pathetically unconvincing measures there are is strikingly uncritical and not even up to date. But his case is even less compelling when he talks about sensitivity, or flexibility or optimism (all of them put forward as neotonous traits), for here he can produce no evidence at all. Too often he is reduced either to stating his own unsupported impressions or to quoting the equally unsupported opinions of other theorists.

Another problem is that Ashley Montagu's (always attractive) enthusiasm for his neotonous case is so strong that he seems unable to notice that his thesis is, as far as several other people's theories are concerned, an antithesis. He gaily quotes Piaget, and yet apparently fails to notice that Piaget spent his whole working life trying to prove the startling intellectual inadequacies of childhood. The contrast between Montagu's optimism and Piaget's pessimism about childish behaviour is stark indeed: I cannot see how they could both be right. But there is no reason why Montagu should be worried by this, since there is now plenty of evidence that Piaget's gloomy stance was, to say the least, exaggerated.

This leads us to another difficulty about the book. Not only does the author skirt round possible arguments against his thesis; he also ignores a great deal of evidence which could be used to support it. The last ten years or so have seen a series of extraordinarily striking experiments which apparently demonstrate that even very young children have intellectual capacities which had been previously thought to come much later on in life. Since this research seems to show how sophisticated and effective even very young children are, it really is right up Ashley Montagu's street. Yet he all but ignores it. So we are left with a book which may indeed be right, but right often for reasons which the author himself leaves out. □

P.E. Bryant is Watts Professor of Experimental Psychology at the University of Oxford.

In between ethology and neurophysiology

R.M. Seyfarth

The Human Primate. By Richard Passingham. Pp.390. Hbk ISBN 0-7167-1356-X; pbk ISBN 0-7167-1357-8. (W.H. Freeman: 1982.) Hbk \$19.95, £14.95; pbk \$10.95, £7.50.

A GOOD science book, one is told, does not just organize and summarize results: it presents a novel synthesis of previous work, offers new hypotheses and points the way to new areas of investigation. This is a difficult task in any field, but it promises to be particularly frustrating when the chosen topics are evolution, the brain, intelligence, and language in human and non-human primates. Not only is this research scientifically complex, but it also has uncomfortable Cartesian overtones, since in the long run it aims to establish a boundary between human beings and other animals. Moreover, there's Catch-22. The more we find human features in the brain or in the communication of non-human primates, the more these animals become ideal models for research on human pathologies. At the same time, increasing evidence of similarities between human beings, apes and monkeys raises serious ethical and scientific questions about the conduct of such laboratory research. The ethical questions are being debated — for example by Marian Dawkins in *Animal Suffering: The Science of Animal Welfare* (Chapman & Hall, 1980). Now Richard Passingham raises some of the scientific issues that are equally worthy of consideration.

How should we characterize a brain that is so much like our own and yet clearly different? Where do the intellectual talents of monkeys and apes end and our own unique abilities begin? Is it wise, given the extraordinary anatomical similarities between ourselves and other primates, to assume a quantal difference between our language and their communication? In addressing these issues Passingham sets himself a considerable task, and it is a testament to his efforts that he partially succeeds.

In my view, the least interesting chapters are those on non-human primate relatives, technology, and culture, in which the author simply reviews areas that are traditionally covered in most biological anthropology textbooks. There are other chapters, however, where the material itself is not new but where the author juxtaposes studies that have not previously been treated together. In Chapter 5 Passingham nicely connects laboratory studies of animal intelligence (usually found in psychology textbooks) with field research on the same species (usually found elsewhere). Similarly, in Chapter 6 he tries (with less success) to link the laboratory "ape language" projects with research on the communication of primates in their

natural habitats. Although such field and laboratory studies seem obviously related intellectually, *The Human Primate* is, surprisingly, one of the first books to treat them together.

This said, novel syntheses can be frustrating because they often reveal more about biases and failures than about insights and successes. Take intelligence: here Passingham reviews the correlation between performance on a variety of psychological tests and at least one index of brain development. To no one's surprise, monkeys and apes score better than other mammals. Insufficient emphasis, however, is placed on the fact that as yet we have no test that reliably distinguishes between the skills of apes and of Old World monkeys. Somehow we *know* that chimps are smarter than macaques, but thus far comparative psychologists have been unable to describe what this actually means. Even when species differences in test performance are found, no successful effort has been made to determine the relation between such scores and the animals' natural social behaviour. For instance while Clutton-Brock and Harvey have shown that primate species with larger brains have larger home ranges, to those interested in how intelligence works and has evolved this remains nothing more than a tantalizing correlation.

Similarly, in other sections Passingham synthesizes two studies on one page, only to adopt a page later the same narrow perspective that has kept them apart for so long. In comparing the "ape language" studies with research on the natural signals of chimps, he correctly notes that evolution is unlikely to have supplied an animal with communicative skills for which it has no need. Thus we should expect, if only as a working hypothesis, to find that free-ranging chimps or other primates are capable of using sounds or other signs to represent objects in the world around them. Passingham concludes too readily that there is no such evidence. The fact is: no one has really looked. For while the ape language projects have begun on the assumption that their subjects are capable of at least rudimentary semantics, observers of the same or related species in the wild have generally not even considered this as a possibility. Given such biases, it is not surprising that few parallels have been found between primates' abilities in the laboratory and their natural communication in the wild. But it would be premature to conclude that no such continuities exist when, until recently, they haven't even been considered.

The Human Primate makes its most important contribution, and has the most to say about the future conduct of research on non-human primates, where it addresses studies of brain mechanisms,

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language and communication. Consider, for example, the search for neural lateralization in non-human primates, or for non-human primate analogues of Broca's and Wernicke's areas in the human brain. Here Passingham's lucid review makes one aware that most research thus far has been a curious combination of highly sophisticated neurophysiology and behavioural assays that are crude and primitive by comparison. When the subjects are human beings, every effort is made to see that they live as normal lives as possible, and that they are tested with meaningful stimuli like words, pictures and other conspecifics. In contrast monkey subjects are often kept in isolation cages and tested with tones, hisses and human speech. Under these conditions is it any wonder that parallels in brain function are often hard to find? Passingham's review offers a challenge for future research, because it shows clearly that good neurophysiology requires equally good ethology. In order to understand the monkey's brain we must also understand — and respect — the social environment in which it has evolved. □

Robert Seyfarth is in the Anthropology Department at the University of California, Los Angeles.

Race is everything

W.F. Bynum

The Idea of Race in Science: Great Britain 1800-1960. By Nancy Stepan. Pp.230. UK ISBN 0-333-28856-4; US ISBN 0-208-01972-3. (Macmillan Press, London/Archon (Shoe String Press), Hamden, Connecticut: 1982.) £20, \$27.50.

IN LIBERAL circles nowadays, "race", if not exactly a dirty word, is at least one which can be vaguely embarrassing. Modern anthropology stresses social forms rather than biological natures as central to the differences between "us" and "them". This was not always the case. As the Scottish anatomist Robert Knox wrote in 1850, "Race is everything: literature, science, art — in a word, civilization, depends on it". To be sure, Knox was a little bit suspect even in his own time, since his religious views were heterodox and he had been the prime recipient of the merchandise purveyed by Burke and Hare. But Knox's racial theories were part of the nineteenth-century fascination with the subject, and not simply as it related to the variations between Hottentots and Englishmen. Anthropology could be studied closer to home: in the differences between the Saxon, who built his detached home in splendid, self-reliant isolation, and the Celt, who swarmed in the slums of

the teeming Victorian cities. Norman yokes and Aryan races were part of the substance of nineteenth-century science.

The title of Nancy Stepan's new book is significant: *Race in Science*. Concentrating primarily on Britain, she traces the rise of the scientific idea of race in the eighteenth century, through its burgeoning in the next century, to its decline in our own. The peripheries are rather fuzzy, for there was a preoccupation with the nature of human differences long before the Enlightenment, and the modern legacy of old-fashioned racial studies (what Stepan calls the "new science of human diversity") is not without its historical reverberations. Not ten years ago Britain's oldest university press published a massive book by a Fellow of its oldest scientific society entitled simply *Race*. Nevertheless, Stepan insists, the

tradition of scientific racism which had lasted from the early nineteenth century until the end of the Second World War if not later, has at long last been laid almost to rest.

The phrase "scientific racism" raises issues which, as we shall see, Stepan is conscious of but does not resolve. But what she does, soberly and cogently, is to describe the idea of race in Britain during the past two centuries. Her book is based on an intelligent reading of the relevant primary and secondary literature, and the result is a thoughtful, well-written synthesis. Three principal themes successively dominated debates on race during the period Stepan covers: the original unity or diversity of mankind; man's evolutionary origins; and the relative eugenic worth of the various human races.

Traditional theological and scientific orthodoxy asserted that all human beings are descended from a single pair, created by God and placed in the Garden of Eden. In the nineteenth century, this monogenist view was most authoritatively developed by the Bristol physician and ethnologist James Cowles Prichard (1786-1848). In a series of erudite books, culminating in the five-volume, third edition of his *Researches into the Physical History of Mankind* (1836-1847), Prichard marshalled the anatomical, physiological and linguistic evidence favouring the original unity of man. His Christian vision inspired many of the founders of the Ethnological Society of London (1843), but by the time of Prichard's death a more secular, continental-inspired polygenism (as represented by Knox) was rapidly gaining ascendancy in British circles. This mid-century polygenism was rooted in the notion of the biological *type* and stressed the permanency of inheritance at the expense of the more fluid environmentalism of earlier naturalists.

After 1859, of course, Darwinian evolution and the contemporary discovery of man's antiquity gave a broader framework for theories of the origin of human beings and human races. Curiously

enough, as Stepan correctly insists (echoing a point persuasively argued by the historian of anthropology George W. Stocking, Jr), late nineteenth-century debates were often couched in roughly the same terms as pre-Darwinian ones. Polygenism persisted long after evolutionary biology might have been expected to have made its traditional formulation irrelevant. Part of the intellectual reason is that typological thinking can be neater and more satisfying than the population or statistical thinking inherent in Darwinian definitions of species and in the Darwinian vision of nature.

Statistics, however, was also central to eugenics, named and inspired by Darwin's cousin Francis Galton. In Britain, eugenicists were generally more concerned with social class than with race, although Stepan deftly picks her way through the eugenics movement, focusing on the all-too-easy transition from class to race. Both eugenics and racial biology in Britain were profoundly affected by the revulsion to Nazi ideas and practices, and the establishment of the neo-Darwinian synthesis by Ronald Fisher, J.B.S. Haldane and Theodosius Dobzhansky gave new sophistication to population genetics at the expense of the typological approach. Since the Second World War, the main arena for these debates has been the psychological laboratory rather than the anthropological field station, as the nature-nurture polarity has revolved primarily around intelligence. In the United States, which at least until the recent past has had a more turbulent racial history than Britain, Arthur Jensen's researches have racial overtones; in Britain, Cyril Burt's data were aimed at showing class differences in intelligence.

Stepan discusses all these issues and more in a book which has many virtues. What she does not do, however, is explore analytically the implications of one of her explicit working assumptions:

that though the connection between racism and science is inescapable, the story of scientific racism is not merely a story of "pseudoscience". Bad science, perhaps, but not pseudoscience [p.xvi].

Her work hints at, but never comes to grips with, the relation between "science" and "prejudice", and between "good" science and "bad" science; with the nature of scientific knowledge; or with those wider issues of class, sex and empire, fact and value, which are central to that telling phrase, *scientific racism*. To write such a book would be vastly ambitious, but it should be possible even within Stepan's own liberal historiographical framework. In the meantime, her present book deserves to be read and pondered by anyone interested in the human sciences or their history. □

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Theoretical archaeology in Melos

Peter Warren

An Island Polity: The Archaeology of Exploitation in Melos. Edited by Colin Renfrew and Malcolm Wagstaff. Pp.375. ISBN 0-521-23785-8. (Cambridge University Press: 1982.) £35, \$65.

FROM 1974 to 1977 the Greek island of Melos was the subject of a multi-disciplinary environmental survey, the fieldwork being closely integrated with excavations at the great Bronze Age site of Phylakopi. An account of these excavations, directed for the British School at Athens by Professor Colin Renfrew, will be published separately. The present volume has as its basis the publication of the survey. There are 15 contributors, J. Cherry, C. Renfrew, B. Sparkes and M. Wagstaff writing major parts. Most of the team involved are or were at the University of Southampton and the volume must certainly rank as a major research achievement of that institution.

The project was concerned with the economic, social and political history of Melos, from the Neolithic period to the present, in relation to the island's whole environment as a resource for exploitation. Geology, geomorphological evolution, agriculture, animal husbandry and mineral exploitation are discussed in detail. The mineralogical studies include reference to millstone extraction, Hellenistic and Roman mining of sulphur and other minerals, and a detailed account by R. Torrence of the celebrated obsidian sources. Her statistical analysis of waste and worked pieces indicates unsystematic exploitation of the sources, with no evidence that Phylakopi controlled an Aegean market economy in obsidian.

Other contributions examine settlement distributions, forms and history (together with an excellent site gazetteer by Cherry), and the changing impact of autonomy and external control on the Melian populations. There is some fine topographical

work by Cherry and Sparkes on the Classical town of Ancient Melos. Wagstaff has a most informative chapter on the relations of mediaeval Melos with the outside world, with invasion maps of Arab and other raiders worthy of a central European *Völkerwanderung* atlas. The same author and S. Augustson examine traditional land use in the island; their interesting data for plot distances and journey times from farms and homes suggest controlling factors specific to Melos, or at least in contrast to those for Crete and Messenia. It would have been helpful if the land-use questionnaires had been included in the book, though the authors do provide much statistical information on the calculation of labour intensity coefficients for agriculture. The text throughout is supported by an extensive range of maps and tables.

Today all this is expected of such a project in early Aegean cultural studies. The account of the University of Minnesota's work in Messenia, *The University of Minnesota Messenia Expedition: Reconstructing a Bronze Age Regional Environment* (1972), is a magnificent predecessor. Current work in the southern Argolid and in Boeotia by American and British teams respectively is on similar, large-scale, multidisciplinary lines.

But here all comparisons stop. The present volume is *sui generis* in Aegean studies. It has a character and purpose far beyond the achievements of the studies so far referred to. The guiding inspiration is Professor Renfrew's. The purpose is not simply environmental reconstruction and explanation of state formation and disaggregation through time; rather it is to use Melos as an instance of the general processes affecting modes of state formation. So the character of the book is largely theoretical, deploying a wide range of models to investigate the central aim.

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The living past on Melos — threshing the harvest in the traditional manner, using two donkeys to pull the threshing sledge (*volosyro*).

At times the text is not easy to follow: detailed modelling of the "intensification of subsistence production" in terms of the "production function" and "the incentive elasticity of labour input" are crucial concepts. A model of "socio-spatio-information hierarchy" for Melian state formation is discussed but rejected. So too are explanations based on irrigation, redistribution, secondary state status, importation of prestige goods and a temple-based system. The explanatory model that is chosen is one of "peer polity interaction". This answers well to *growth* in the economies and societies of the Aegean city states, growth through continuous economic exchanges between the states, from their differing resource bases. But I am not sure that it explains *initial* state formation. In Renfrew's final chapter the foundation of Classical Melos by Dorians emigrating from Lakonia, discussed earlier in the book, is strangely neglected.

My own view, which I am confident by no means all Aegean archaeologists will share, is that this book will have an important (and perhaps divisive) influence on future Aegean studies which attempt to go beyond the descriptive and try to tackle economic and social questions. It therefore merits long and serious study, even if one feels that what are proposed in the introduction as general propositions and hypotheses which "have dictated the final form of the book" seem either so general as to be unhelpfully obvious (resource potentials will support a range of population densities) or look rather like inferences after the fieldwork was done. But any big field project will develop its aims as it goes along; it responds to observed data. There is certainly no harm in that. □

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Times. In contrast the author gives a sympathetic account of the rather pathetic antiquities dealer, Shapira, whose almost certainly genuine "Dead Sea Scroll" fell prey to the same Clermont-Ganneau, an experience which was to lead eventually to the poor man's suicide. Even some of the best forgotten exploits are enthusiastically remembered by Silberman, such as Seetzen's ill-prepared and disastrously executed survey of Eastern Palestine or Montague Parker's outrageous search for the treasure of Solomon beneath the Dome of the Rock in Jerusalem.

Much attention is rightly given to the outstanding and pioneering work of the Palestine Exploration Fund. It was this



Frontispiece of C. Conder's *Tent Work in Palestine* (1878).

organization more than any other which changed the course of exploration in the Holy Land, removing it once and for all from the hands of the wealthy dilettante and firmly establishing it as an academic and scientific discipline. Today, Palestinian archaeology is the most sophisticated of all the branches of Near Eastern archaeology, with refined stratigraphic excavation techniques and pottery chronologies capable of differentiating between periods as little as 50 years apart. Silberman successfully shows how the groundwork for these achievements was laid: ironically perhaps, two of the great innovators, Sir Flinders Petrie and George Reisner, were "borrowed" from Egyptology.

Relevant and interesting illustrations have been chosen to accompany the text, and the map showing the location of the various late nineteenth and early twentieth century excavations is a most useful adjunct. Altogether, this is an excellent book which can be recommended to both specialist and non-specialist. For students of Near Eastern archaeology, it must be placed in the category of essential reading.

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Politics, personalia and the past

Jonathan Tubb

Digging for God and Country: Exploration, Archeology, and the Secret Struggle for the Holy Land 1799–1917. By Neil Asher Silberman. Pp.228. ISBN 0-394-51139-5. (Knopf: 1982.) \$16.95.

THERE are many people whose radios never change from a station broadcasting popular music yet will avidly read books on the life of Vivaldi or the extra-marital affairs of Alban Berg and will derive great enjoyment from them. The point of making this statement is simply to emphasize that it is not necessary to be a Palestinian archaeologist, or for that matter an archaeologist of any sort, to appreciate Neil Asher Silberman's remarkable book.

Unlike other authors who have charted the progress of exploration in the Holy Land, Silberman has gone much further, not only chronologically by extending the story back to the exploration by Napoleon Bonaparte's "Scientific and Artistic Commission", but also in manner of presentation. He has avoided a tedious procession of facts about who dug what, where, when, and what they found, and instead presents a series of fascinating insights into the various researches seen in the context of the prevailing socio-political situation.

For example, not only does the author describe in great detail the complicated events which followed the discovery of the Moabite stone, events which finally led to the tragic destruction of the monument, but adds a new dimension by setting the whole sad saga against the backdrop of the equally intricate international relations

among Great Britain, France and Germany just prior to the Franco-Prussian war. The near frenzy which accompanied the desires of the various scholars to acquire this monument is explicable in terms of its outstanding importance. For the black basalt stela, discovered at the site of biblical Dibon, commemorates the successful attempt of Mesha, King of Moab in the ninth century BC, to liberate his country from Israelite domination. It was, therefore, one of the first and most spectacular examples of an ancient inscription corroborating a biblical narrative, for the same events, albeit with a changed nationalistic bias, are recorded in the Second Book of Kings (II 3: 4-27).

So too, Silberman helps us to understand that the series of really massive excavations conducted by the *Deutscher Palästina Verein* during the early years of this century, e.g. Sellin at Ta'anach, Schumacher at Megiddo, and Sellin and Watzinger at Jericho (Carl Watzinger was the co-director at Jericho, not Schumacher as stated by Silberman), were conceived as part of a deliberate German policy to support and strengthen the failing Ottoman Empire as a buffer against its fragmentation at the hands of the British, French and Russians.

Ultimately, however, this is a book of personalia and Silberman paints vivid, if not always flattering portraits of many of the giants of the subject. Charles Clermont-Ganneau, the brilliant French archaeologist and scholar, comes off worst, depicted as the sort of man to whom one would not tell the time of day for fear of it being denied and ridiculed in the

Variations on the theme of cartography

Sarah Tyacke

Early Thematic Mapping in the History of Cartography. By Arthur H. Robinson. Pp.266. ISBN 0-226-72285-6. (University of Chicago Press: 1982.) \$35, £24.50.

OPEN any atlas today and the chances are that many of the maps you see will be thematic, that is they show the distribution of some particular phenomenon stretched across a base map. The themes may range from the physical structure of the world to any aspect of human life, including abstract concepts; they are mapped by a number of graphic methods, immediately understandable by almost everyone, such as dots, flow lines, isolines and various types of tonal shading. These cartographical commonplaces were rarely, if at all, in evidence before 1700 and only took off in the first half of the nineteenth century. Neither the idea of thematic mapping nor the techniques for doing it were widely disseminated in intellectual and other educated circles of earlier times.

As the result of many years of research, A.H. Robinson has recovered the history of thematic mapping and vividly recounted its tender early years and its remarkable flowering under the impact of official and other statistical gathering from the end of the eighteenth century onwards. Unlike the general history of cartography, Robinson has few predecessors in this field and has provided *ab initio* the framework into which the maps gleaned from the works of statisticians, medical doctors, geologists, administrators and others may be slotted.

Although such maps are now very much the province of geographers and allied professionals, the earliest maps, so termed, were more usually drawn to illustrate or to analyse particular distributions or correlations between phenomena and the countries concerned by specialists in other fields; as such the maps are not to be found in atlases but are attached to official reports and professional papers, and illustrate a variety of subjects. For example, medical statistics on cholera were transformed graphically by Dr John Snow in 1855 to demonstrate the relationship between contaminated water supplies and the disease in London: it is these types of maps that Robinson describes and illustrates in the book.

Robinson has felt obliged to narrow his view of thematic mapping, however, to what he regards as the thematic map *sensu stricto*:

ideally the thematic map has as its purpose the display of the overall structure or meaningful character of a distribution rather than simply showing where some things are.

In so doing — and additionally because he does not see some classes of thematic map as contributing to the development of graphic means of representation of data on

maps — a number of types of thematic maps are not considered: for example, land use maps and other categories which may perhaps be called "special purpose" maps are not included. In the latter grouping might be mentioned such items as military plans, road maps and ecclesiastical maps. These types may or may not differ in graphic representation from that found on topographic maps but their function is primarily for a specific purpose and often illustrates a theme. In the case of ecclesiastical mapping a typical thematic example would be an atlas describing the numbers and distribution of properties owned by the Capuchin monks dated 1712. A key to the numbers of monasteries, priests, monks and lay brothers accompanies each map. The idea of representing the numbers by some manner of proportional symbolism is not present but the thematic element is quite obvious in this type of map.

Although it would indeed seem to be the case that the introduction of various thematic maps and symbols did not, as Robinson shows, occur before the end of the seventeenth century, it is perhaps unwise to assume that no one had previously conceived of the idea of depicting the structure of one phenomenon on a map. Certainly such an idea was not at all common but it is evident, even from some sixteenth century texts and maps which survive, that at least some people came close to the idea; for instance Queen Elizabeth I's minister William Cecil drew a map of the south-west of England and Wales showing the distribution of copper and lead mines in the 1570s. If one can see in this and similar attempts appreciation of some of the practical uses of such mapping, it must also be admitted that these maps show the qualitative location of phenomena rather than their quantitative distribution. It is quantitative mapping and its methods of representation which, as Robinson rightly points out, only emerge in the first half of the nineteenth century as a response to statistical studies.

The book is completed by 110 illustrations, many coloured, which are for the most part models of reproduction. In particular the selective use of legible detail rather than the reproduction of entire maps, which are so often indecipherable, is most welcome. Beyond this the great value of Robinson's work is that he has not only found a great many examples of thematic mapping, however defined, across a wide spectrum of professional endeavours, but has then produced a convincing and illuminating history which may be truly regarded as seminal. □

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Lamarck the Mythical Precursor

A Study of the Relations between Science and Ideology

by Madeleine Barthélemy-Madaule

Translated by Michael Shank, this book presents a highly readable account of Lamarck's theories and the debates they generated. He emerges as a bold and intellectually adventurous pioneer whose early work centred on meteorology and botany and who became the leading authority of his period on invertebrates. The author does not attempt to rehabilitate Lamarck, but shows that his theories are still relevant to the debate on innate versus acquired characteristics. November 1982, £12.25.

When the Snakes Awake

Animals & Earthquake Prediction

by Helmut Tributsch

Tributsch has collected and evaluated reports on 78 earthquakes from ancient times to the present, covering a broad geographic and cultural range. His book describes the unusual behaviour of animals in the minutes, hours or days before an earthquake struck: some of these connections, long dismissed as folklore, have been confirmed in the scientific literature of the 1970s. Tributsch presents an hypothesis to account for conditions that might trigger abnormal animal behaviour before an earthquake, and reports on such other premonitions as clouded springs, strange fogs, and enigmatic luminous phenomena. Translated by Paul Langner. November 1982, £14.00.

Science & Technology Centers

by Victor J Danilov

Science and technology centres alive with moving gears and levers, activated computers and meters, flashing strobes and lasers, and other participatory exhibits, are designed to explain scientific concepts and technological artifacts. This book, by the president and director of the Museum of Science & Industry in Chicago, consists of practical advice from an enthusiastic promoter of the concept, illustrated with numerous examples of successful programs initiated by centres throughout the world. November 1982, £28.00.

Abusing Science

The Case Against Creationism

by Philip Kitcher

"... a readable overview of modern evolutionary theory, of the facts that support it, and of the various points of scientific controversy. However, his main effort is to examine and refute the creationist argument in detail. Readers... may want to follow up some of the extensive references the author gives to creationist literature. I doubt, however, that this would do more than reinforce the author's conclusion." — *Christian Science Monitor*. October 1982, £10.50.

Nature's Second Kingdom

by Francois Delaporte

"[Delaporte's] project is to chart the concepts and practice of 18th century discourse on 'vegetality'... He shows how these were related to questions about the nature of life and, most important, he argues that the epistemological standards of knowledge about plants was *animal* physiology." — *Annual of Science*. Translated by Arthur Goldhammer. May 1982, £14.00.

Genetic Alchemy

The Social History of the Recombinant DNA Controversy

by Sheldon Krimsky

This book offers a probing analysis of the setting for the rDNA debates, the postulated biohazards, the risk assessment process, the politics of science and regulation, and the means through which biologists protected their discipline. October 1982, £17.50.

The MIT Press

The Massachusetts Institute of Technology,
126 Buckingham Palace Road, London SW1W 9SD.

Changing visions of an ancient society

Arthur A. Demarest

Ancient Maya Civilization. By Norman Hammond. Pp.337. Hbk ISBN 0-521-24017-4; pbk ISBN 0-521-28399-X. (Cambridge University Press/Rutgers University Press: 1982.) Hbk £22.50, \$27.50; pbk £7.95, \$12.95.

A MILLENNIUM ago the dense Peten jungle of Central America swallowed up many of the sprawling cities of the ancient Mayas, entombing their lofty temples, elegant palaces and graceful sculptures beneath a blanket of vegetation. During the past century and a half archaeologists, scholars and adventurers have spent much of their lives trying to unravel the mysteries of this lost civilization. *Ancient Maya Civilization* by Norman Hammond of Rutgers University is the most recent presentation of their findings and of our understanding of ancient Maya history and culture.

The Maya-speaking Indians of eastern Mexico and northern Central America have a heritage that can now be traced back for over 3,000 years. It was, however, the Classic Period (AD250 to 900) that witnessed the florescence of Maya culture — leaving a legacy of hundreds of archaeological sites in the jungles of northern Guatemala, Belize and the Yucatan peninsula. During this golden age, great centres such as Tikal, Palenque and Uaxactun flourished in an environment which appears utterly inhospitable to European and North American eyes. The hallmarks of this period are its fine masonry architecture, monuments sculptured in delicate bas-relief, raised stone roads and polychrome-painted ceramics whose beauty rivals the vase painting of Classical Greece. The sophistication of the Classic Period of Maya culture can be seen in its fully developed writing system, only partially deciphered, which records calendrical calculations and astronomical knowledge of astonishing accuracy — all based solely upon relentless non-telescopic observation and recording. Despite these cultural achievements, the great jungle cities of the southern zone of Maya culture were abandoned some time about AD900. Archaeologists have suggested various causes for the fall of the southern lowland centres including invasion, disease, ecological mismanagement, peasant revolt or complex combinations of such factors. Yet no consensus has been reached and this collapse remains a great mystery.

Hammond's *Ancient Maya Civilization* could not be more timely, since it comes fast upon the heels of a revolution in Maya archaeology. For half a century, scholars have understood the general nature of the Maya intellectual achievements. These have been beautifully described in the classic works of great Mayanists such as J. Eric S. Thompson and Sylvanus Morley. However, we can now see that these

scholars misunderstood many fundamental aspects of Maya culture. The Maya were characterized as isolated, peaceful, theocratic societies supported by small populations of peasants using simple slash-and-burn agriculture and governed by priests concerned only with the worship of time and with astrological lore. Hammond's book incorporates the most recent research on settlement, agriculture and hieroglyphics, which shows Classic Maya society to have been far more dynamic than previously believed. Breakthroughs in hieroglyphic interpretation have revealed that the Classic Maya political landscape consisted of petty lords battling for control of regional city-states. Meanwhile, large-scale archaeological surveys have uncovered complex agricultural systems — raised fields, canals, terracing and other means of water control — which supported dense (proto-urban) populations.

Hammond artfully combines the knowledge gained by the early scholars with the most recent interpretations. First, he draws in the reader unfamiliar with the subject with dramatic, at times romantic, chapters on nineteenth-century explorations of the Maya ruins and twentieth-century archaeological projects. The following, more substantive, chapters on Maya ecology and cultural history are rendered accessible by numerous illustrations, maps and charts. The tone then shifts in thematic chapters on subsistence, politics and trade, in which Hammond presents the evidence for the new, more realistic, characterization of ancient Maya society. Yet in the subsequent accounts of Maya architecture, art and religion, he draws heavily upon the works of the early Mayanists with an undisguised admiration. Hammond also maintains a balance in his discussion of still controversial topics, such as specific interpretations of Maya dynastic history, foreign influences on the Maya and the causes of the Classic Maya collapse. In short, the author is surprisingly successful in integrating old and new conceptions of the ancient Maya.

The book, however, is less successful in dealing with the regional diversity of the civilization. Hammond himself is an active field archaeologist, excavating in Belize, and his text reflects a regional bias towards the southern, lowland manifestation of Maya culture. In the chapter reviewing the rise and fall of Maya civilization, he gives only the most superficial treatment of the development of the great centres in Yucatan, the Guatemalan highlands in the south, and the south-eastern frontier in El Salvador and Honduras. In the thematic chapters, Hammond similarly draws most of his examples from the same handful of sites in Belize and the southern lowlands. Students and laymen reading Hammond's outline of Maya history also should be

made aware that his very early datings for the beginnings and development of complex village societies in the lowlands (which I personally accept) are still being hotly debated.

These criticisms notwithstanding, the book is an excellent introduction to the ancient Maya. Indeed, even the author's research biases, when combined with his crisp journalistic style, help to convey the fluid nature of Maya archaeology in which interpretations are undergoing constant revision as each excavation unearths new contradictions and fresh controversies. Hammond's book achieves a unique fusion of a reverence for the early archaeologists and explorers with an insistence on the most current interpretations. It is a perspective which gives the reader a new vision of both this lost civilization and the volatile discipline that is rediscovering it.

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A ring of irony

J.W. Cornforth

The Enchanted Ring: The Untold Story of Penicillin. By John C. Sheehan. Pp.224. ISBN 0-262-19204-7. (MIT Press: 1982.) \$15, £10.50.

IN 1941, when Howard Florey's team at Oxford had demonstrated the value of penicillin for treating bacterial infections, it became imperative to find better ways of producing the drug in quantity. A huge cooperative effort by 39 industrial, university and government laboratories in the United Kingdom and United States explored not only the existing method of fermentation, but also the chemistry of penicillin in the hope of producing it by synthesis if fermentation techniques could not be improved. Research on the fermentation process was brilliantly successful, and the problem of producing penicillin was solved. In contrast, although the chemical effort succeeded in finding out the structure of penicillin it failed to find an efficient method of synthesis.

As the urgency of the problem faded, chemists engaged on the synthesis of penicillin turned to other work. The most notable exception was John Sheehan, of Max Tishler's team at the Merck laboratories. Leaving Merck for the Massachusetts Institute of Technology after the war, Sheehan sought a rational synthesis of penicillin and in the end he found one. His book is partly an account of his personal involvement with the penicillin problem and partly an anecdotal story of the drug's discovery and development. It is difficult to picture the readership at which Professor Sheehan is aiming; but the book

will certainly interest, and probably irritate, all those who have worked on penicillin.

Sheehan's account of the early days of penicillin reads largely as an attempt to magnify Fleming's contribution at the expense of Florey's and Chain's. He throws no new light on this old controversy. More novel is his account of the organization behind the research and production effort in the United States in 1941–1944. He draws here on minutes of the US Committee on Medical Research, and other official papers. He has recorded on tape the reminiscences of some principal participants, notably Karl Folkers, and he reproduces extracts from these, along with an account from Hamao Umezawa of a belated Japanese effort to produce penicillin in 1944–1945. The absence of chronological order makes difficulties for the reader, especially in a book so obsessed by priority and credit. The difficulty persists throughout.

The part of the book dealing with chemistry is in many ways the most disappointing. Chemists are likely to be put off by the unexpectedly poor appearance and layout of the diagrams, and by oversimplification that sometimes sacrifices truth, whilst the non-chemical reader may well be confused and misled. This is particularly true of the chapter where one would expect it least — the description of Sheehan's own work. A brief account, based less on the book than on the scientific literature, seems indicated here to make clear Sheehan's contribution.

The molecule of penicillin is relatively small and simple, and the difficulty of synthesis arises mainly from the presence of several reactive groupings that cannot easily be manipulated without mutual interference. It was already recognized by the end of 1943 that penicillin is an anhy-

dride of penicilloic acid, a substance easily made from penicillin and easily synthesized. A decision between two possible structures for this anhydride was eventually made in favour of the β -lactam structure, but it was found to be impossible to produce this from penicilloic acid or its simple derivatives. That was the problem that Sheehan solved. His earlier successes were obtained by making inert analogues of penicilloic acid that enabled him to use a quite vigorous reagent — thionyl chloride — to close the strained β -lactam ring. The breakthrough came when he discovered that carbodiimides could be used to form amides in mild conditions from carboxylic acids and amines; a most valuable peptide synthesis arose from this finding. The cyclization of penicilloic acid to penicillin is a special type of internal peptide synthesis, and when the carbodiimide method was applied to a suitable penicilloic acid a natural penicillin was formed in low yield. This work was submitted for publication in February 1957.

Sheehan's method for synthesizing penicilloic acids proceeded by way of an analogue lacking the acyl side-chain characteristic of penicillins. He tried the long shot of cyclizing this compound and obtained a crude mixture containing a very small proportion of racemic 6-aminopenicillanic acid (penicillin without the acyl side-chain). When this mixture was treated with an appropriate acyl chloride, formation of a penicillin could be shown to have taken place. Sheehan did not publish this experiment but he applied for a patent on it, in March 1957. In 1959, he modified the process by using a trityl group to protect the reactive amino group before cyclization; he then obtained much better yields in closure of the β -lactam ring, though the yields from subsequent removal of protecting groups were poor. This procedure gave him fully synthetic 6-aminopenicillanic acid.

In 1957, workers at the Beecham Laboratories isolated 6-aminopenicillanic acid in pure form by fermentation. This completely independent discovery was the basis for an enormous development of "semi-synthetic" penicillins carrying "unnatural" acyl side-chains. That whole development rests on aminopenicillanic acid derived from fermentations and no penicillin has been produced in commercial quantities by total synthesis. Nevertheless, Sheehan's commercially valueless procedure enabled him to gain, after a protracted legal battle, priority of invention. The last chapter in his book describes that battle.

Sheehan's achievement in making a total synthesis of penicillin was a splendid piece of work. His book, in contrast, lends its own sub-title — *The Untold Story of Penicillin* — a ring of irony. □

Sir John Cornforth is Royal Society Research Professor at the University of Sussex. He is a past author of *The Chemistry of Penicillin* (Princeton University Press, 1949).

Children of clay?

N.W. Pirie

Genetic Takeover and the Mineral Origins of Life. By A.G. Cairns-Smith. Pp.477. ISBN 0-521-23312-7. (Cambridge University Press: 1982.) £15, \$29.50.

EMINENT physicists have written so much cocksure balderdash about the nature and origins of life, that it is reasonable to be apprehensive about a contribution to the subject from a chemist. We have become accustomed to the naive assumption that, because life is now dominated by the behaviour of proteins and nucleic acids, a study of the conditions necessary for their probiotic synthesis and interaction is relevant. (If that assumption were valid, the right approach to the history of the use of fire would be to study the mechanism of a cigarette lighter, and a study of the composition of paper and ink would illuminate the history of writing.) Cairns-Smith argues instead that proteins and nucleic acids were successful late arrivals on the biological scene. This is an unpopular, but not wholly novel, point of view — a few of us have been adumbrating it for many years. What is novel is that he suggests a concrete alternative hypothesis, almost literally concrete because he gives silicates a fundamental role.

The argument is that clay minerals are not simple rock fragments, but are recrystallized structures with a final form which depends on the conditions of crystallization, the extent to which supersaturated solutions are seeded and the transmission of accidental defects. The last point will be the most controversial. An evolving quasi-biological system needs a genotype and phenotype: this is the old distinction between form and substance. In biology, contrary to Aristotle's dictum, it is the form, the genotype, that lasts and not the substance.

About a quarter of the book is occupied by a description of the immense variety of clay minerals, the distortions and defects in them, and their behaviour in the presence of other substances. The photomicrograms and electronmicrograms show complicated folded laths and other structures, colourfully described as "micro-origami", "card houses" and "a self-assembling maze". Even without the detailed and plausible argument which is presented here, the structures shown in these illustrations support the idea that, if prevital evolution happens anywhere, this is a reasonable group of substances within which to search for it. The old analogy between life and crystallization, which was discussed by Kant, Reil, Troland and others, may now legitimately be elevated from the status of analogy to that of hypothesis. Nevertheless, it may be argued that the mechanism suggested — for example a particular type of clay crystal perpetuating its ability to thrive in crevices

Elks, Whelks, and their ilk

*THE monarchs of the Irish bogs
Succumbed to neither men nor dogs*

But (most ecologists agree)

To calcium deficiency.

*They scoured the base-deficient peat
For antlers and old shells to eat*

And, finding all too few, they died

Around the Celtic countryside.

...
*Then mourn the passing of the elks,
But note the wisdom of the whelks*

*That roam the shore — their
native heath*

With silver-indurated teeth

*And bore to death their mollusc friends,
Who come to sad, unsuccoured ends.*

Without the need for gins and limes,

The whelks survive to modern times.

...
Thus ungulate and gastropod,

*And all that live by sea or sod,
Are doomed to be — or not to be*

By biogeochemistry.

Ralph Lewin

in sandstone by shedding "seeds" into neighbouring crevices — is rather remotely connected with what we normally regard as life. That objection applies with equal force to the organic polymerization picture which has recently become conventional. Neither picture fills the gap between the never living and the nearly living in a wholly satisfactory manner. But it is pleasant to have a change of outlook.

Cairns-Smith chooses clay minerals as the vehicle for biopoesis because of their versatility and indubitable abundance on the probiotic Earth. However, evidence from meteorites, and from *in vitro* experiments going back to the time of Berzelius and Berthelot, make it equally certain that tars would also be present. Tars are the main product of the tediously repetitious experiments in which gas mixtures, made up according to constantly changing opinions about the composition of the probiotic atmosphere, are now exposed to various types of energetic insult. Tars are mentioned here several times. They are invariably disparaged as inert end-products or as "tarry chaos". I have been arguing for many years that sunlit tar is as likely a site as any other for an early stage of biopoesis, but after reading this book I would include clay minerals in the notional mixture and, following Goldschmidt, some minerals containing the transition elements. Everyone to his taste. Cairns-Smith is a clay enthusiast: he enjoys giving clay a role more dynamic than mere adsorption and sequestration.

There is no suggestion here that clay filled this dynamic role in the hypothetical eobiont any better than protein would have done. On the contrary, there is the explicit statement: "Anything clay could do organic molecules would eventually come to do better". To quote again, the discussion is said to be about: "What was biochemistry like when protein was an optional extra?". Several passages emphasize the point that eobionts need not have been small. The idea that life started small presumably stems from a fallacious conflation of early life with viruses. Being obligate parasites, viruses have obviously nothing to do with the case: it is not even useful to classify them as organisms. It is only when competition with other organisms puts a premium on rapid reproduction that smallness becomes advantageous. In the beginning there was no competition. As Cairns-Smith puts it:

The microscopic size of the modern bacterium should be seen as one of its most notable achievements rather than any sign of evolutionary infancy. The bacterium is a highly concentrated package of ingenuity.

If minerals have the potentialities attributed to them here, it should be possible to demonstrate processes comparable to evolution taking place among them now. This point is discussed briefly with references to some as yet unconfirmed claims that such processes occur in various

precipitates. It is not enough to show that there are progressive changes in these precipitates; to be convincing it should also be demonstrated that the change is hastened when a fresh precipitate is inoculated with a "seed". The absence of evidence for this does not invalidate the hypothesis. About a gillion (10^9) years seem to have elapsed between the time when physical conditions on Earth became comparable to those with which we are familiar, and the formation of the first organisms. Relevant *in vitro* processes need not therefore be rapid.

Evidence that the first organisms were inorganic could also come from an appraisal of the present-day biological role of minerals. Some organisms show great enterprise in exploiting specialized properties such as the magnetism and hardness of Fe_3O_4 and the density of BaSO_4 . It has been suggested that the five-fold symmetry of most echinoderms is imposed by the 75° angle between the faces of calcite crystals, and it may be legitimate to wonder whether the greater intracellular importance of K rather than Na is a relic of the behaviour of these ions in clays. On the whole however, such typical mineral elements as Al and Si seem to have been steadily eliminated. It is primitive plants that make most use of Al, and primitive animals of Si — though there are still substantial amounts, with obscure functions, in the human body. Clearly, evidence for a

mineral background to biology is tenuous: the postulated takeover is nearly complete. We are, on the whole, reasonably satisfied with the result. Sir Walter Scott's words are apposite and partly justify the title of this review: "Well hast thou done, frail child of clay!"

Recent articles and letters to the Editor of *Nature* have shown that there is disagreement about the principles that operate and have operated in evolution. The theme often crops up in this book. At a time when we are still arguing about the survival value of many queer features in living organisms, some of the statements made have a rather over-confident tone. It will be interesting to see what comments on that aspect of Cairns-Smith's thesis are made by reviewers with a primary interest in evolution rather than chemistry. Cairns-Smith is commendably tentative, he recognizes that a crystalline gene might not work and says that: "... when we are near to the answer we will find many similar answers, and so we will not be able to say exactly how life must have started on Earth". He writes clearly with many neat phrases and some humour. The result is an excellent book — the most plausible and sensible that I have seen on the subject. □

N.W. Pirie was formerly Head of the Biochemistry Department at Rothamsted Experimental Station, Hertfordshire. He has been writing articles on the nature and origin of life since 1937.

How to get the wind up in the future

F.J.P. Clarke

Wind Energy for the Eighties: A Review by Members of the British Wind Association. Pp.372. ISBN 0-906048-73-7. (Peter Peregrinus: 1982.) £19.50, \$49.50.

Is wind energy a credible source of electricity for the world, and in particular for the UK? *Wind Energy in the Eighties*, a status review written by experts from the British Wind Energy Association, gives a clear answer: wind energy could readily contribute 20 per cent of the UK's electricity supplies and it could be well on the way to that by the year 2000. How valid is that claim?

Although detailed resource surveys have still to be carried out both on land and offshore, the book explains why resource size is unlikely to be a limitation. Because the wind is an unreliable source of power, our electricity system is limited in what it can accept to perhaps 20 per cent of total demand. But that much at least the Electricity Boards agree can be accepted; and since the feasible resource base is likely to be much greater than that, resource size is unlikely to be limiting — provided, that is, the economics are right.

There are two aspects to this latter

question, the value of wind power and its cost. Because of the unreliability of wind power, its value must be judged primarily in terms of the conventional fuels it displaces when the wind is blowing, which depends on the plant mix — how much coal or oil will be saved? And that depends on how many wind machines are in the system; the more wind machines there are, the smaller is the value of the next machine to come on stream because the highest running-cost conventional plant will always be displaced first. Modelling such system economics is difficult; present models may be inadequate, and the fact that one is concerned with a plant mix well into the future adds to the difficulties. Nevertheless estimates can be made and are given in the book, assumptions being clearly stated.

The second aspect of the economics is the cost of the wind machines themselves and their associated plant. Here, overseas estimates tend to come out lower than those made in the UK. While this may in part be the problem of relating US dollars or Swedish kroner to British pounds, the book does point up differences in technical assumptions that also contribute.

From all these facts, speculations and assumptions the balanced conclusions emerge that wind machines can be integrated into the electricity grid in substantial numbers, and that in some cases it should be economically worthwhile to do just that.

If these conclusions seem modest against the claims sometimes made for wind power, it should be remembered that they are formulated by professional scientists and engineers who are as aware of the uncertainties as of the very real promise of the technology. More knowledge is needed to lower costs and encourage adoption of the technology, especially offshore.

One of the important concepts behind the possibility of lowering costs is the compliant structure. The design approach in the UK has so far been based upon structural stiffness to avoid resonance and vibration which are notoriously difficult to predict, and which can be so damaging. Such thinking was also behind the earlier US designs. However, subsequent US, Swedish and German designs have gone for a more compliant structure. This involves a much better understanding of the dynamics of the whole structure so that lighter, smaller and less-expensive

components can be used. The dynamic forces can then be absorbed with compliance rather than with stiffness. As is admitted in the book, this approach is rapidly gaining favour as an important way to reduce costs, though the extent to which the concept can be applied in the highest wind regimes in the UK has still to be determined. However, as reflected by the cursory treatment, the UK is lagging in this area.

Two basic types of machine are being developed industrially in the UK and both receive good coverage together with their overseas counterparts. The horizontal axis machine had the earlier start, and this has been followed by the vertical axis concept. The rotor blades of horizontal axis machines are subject to reversing gravitational loadings as they turn, thus making them the components most at risk from fatigue failure. The vertical axis machines avoid this and have the additional advantage of being omnidirectional; however they would be more prone to other problems which need investigation.

At megawatts size either machine would look large alongside the traditional windmill and could reach higher than the largest electricity pylons, thus raising the problem of visual amenity. Also, there have been reports from the United States of television interference, windows rattling and other environmental impacts; but so far they seem to be rather isolated events. On this type of issue, as on technoeconomic issues, real answers will come only when large machines are up and working in the UK.

Such possible environmental problems make it attractive to place machines in shallow waters offshore. This would cost more, but, it is claimed, expense may be outweighed by the higher wind speeds and the environmental freedom to build bigger structures. Whatever these pros and cons, different practical and institutional advantages are also covered in the book. Wind machines of 1–10MW provide a modular power source that can be added to the system quickly as needed, without all the well-known problems of massive construction projects. In addition, it is pointed out that such machines could provide the beginnings of decentralized power supplies — a possibility that could attract support across political boundaries.

Wind Energy for the Eighties brings together professional views from the academic world, from industry both public and private, from research institutions and from countries overseas. Apart from its value as a status report, the book sets out simply and clearly the background theory to many of the topics. Students and those new to wind energy will find it as good a source book as those already in the field. It is a pleasure to welcome it. □

F.J.P. Clarke was Chairman of the National Wind Energy Steering Committee until 1981. He is now Director of Strategic Studies at the United Kingdom Atomic Energy Authority.

Cornucopian faith

Eric Ashby

Catastrophe or Cornucopia: The Environment, Politics and The Future. By Stephen Cotgrove. Pp.154. Hbk ISBN 0-471-10079-X; pbk ISBN 0-471-10166-4. (Wiley: 1982.) Hbk £16, \$35; pbk £6.95, \$15.30.

OVERHEARD in a pub, two women talking: "She was as stubborn as a mule. But I was just as firm". This is the theme of Professor Cotgrove's book: the controversy between those he calls catastrophists and those he calls cornucopians, those who predict a collapse of industrial society and those who believe that threats to our survival will continue to be kept at bay by the very products of that society. Each side so sure it is right and so ready to dismiss the other side as irrational. Each side having access to the same set of facts but interpreting them so differently.

Professor Cotgrove is a sociologist who believes in using quantified data prepared from questionnaires for an analysis of what a layman would call "public opinion" on environmental issues. His aim is to discover what sort of people are catastrophists, and what sort cornucopians. He wisely discards these picturesque (and in my view misleading) labels for more specific categories: environmentalists (a sample of 576 persons from the Conservation Society and the Friends of the Earth), industrialists (a sample of 400 from *Business Who's Who* and *Who's Who of British Engineers*), trade union officials (399 names from the *Trade Union Handbook*), conservationists (500 names from the World Wildlife Fund, UK Branch), and, to represent the public, 1018 names drawn from the electoral registers of Bath, Oldham and Swindon. These people were asked to respond to a battery of questions about their attitudes to the environment, to various other social issues, to the effectiveness of the present political system, to the morality or otherwise of the imperative of economic growth. With this background of information Professor Cotgrove pursues his aim, concentrating largely on environmentalists on the one hand and industrialists on the other.

The initial results cannot be described as surprising: 92.4 per cent of environmentalists consider environmental problems to be "extremely" or "very" serious; only 45.9 per cent of industrialists share this view. Each category of the public behaves predictably. The environmentalists were selected from a sub-group which has deliberately carried its campaign into politics; belief is often hardened into ideology. Conservationists are milder people, less politicized, inclined to give a higher priority to law and order and to the economy than they do to environmental issues (except the conservation of the species to which they are attached: birds, or whales, or redwoods). Industrialists

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The shape of wind machines to come — an artist's impression of the wind turbine to be built on Bugar Hill, Orkney, by the Wind Energy Group. Overall the structure is 75m in height, taller than Nelson's Column in London.

naturally put their faith in the creation of wealth and tend to underplay anything that threatens that faith. Trade union officials find themselves, as one would expect, sitting on the environmental fence. Of course they want improved social conditions, less pollution, public finance for environmental tasks, but when it comes to jobs versus the environment one doesn't need Professor Cotgrove's statistics to provide an answer.

After this somewhat pedestrian start the book gathers pace. Environmentalists and industrialists emerge as the personifications of two opposing life-styles which Professor Cotgrove (unwisely, I think, for it begs the \$64 question in the book) calls "paradigms". The typical environmentalist is in early middle age, well educated, engaged in what industrialists would call a non-productive occupation (as teacher, social worker, academic, doctor), left wing in politics (unlike conservationists, who are commonly right wing), hostile to the current enthusiasm for economic individualism and commitment to economic growth. The typical industrialist in the sample is over 40, also well educated, engaged in production or marketing of commodities, right wing in politics and living — except in his home life: the book makes that distinction — by the strenuous survival standards of the market place.

In this crude classification some truth, of course, is lost. If environmentalists and industrialists were victims of their respective paradigms there would be no cross-fertilization of ideas between them. It's not as bad as that. It was the industrialist who successfully flooded Latin America with mass-produced automobiles, Aurelio Peccei, who founded the Club of Rome and has become the high priest of environmentalists. So not every reader will agree with Professor Cotgrove when he says:

Industrialists and environmentalists . . . inhabit different worlds. From where they each stand, the world looks different. What is rational and reasonable from one perspective is irrational from another.

It is a caricature, but the merit of caricatures is that they do carry some truth. It is not necessary to swallow the whole of Professor Cotgrove's thesis in order to get from him some useful, if uncomfortable, ideas. The idea that strikes me as most important is the suggestion that the issues environmentalists get so worked up about — acid rain, nuclear waste, lead in petrol, draining of wetlands — are symbols of a much deeper rejection of the social order that finds it fitting to exploit the environment in these ways. Accordingly, though the industrialist and the environmentalist might agree on the desirability of taking lead out of petrol, they would act for totally different reasons: the one, out of expediency, the other as a step toward ridding the air of a moral, as well as a material, impurity. The book

quotes the well known work by Mary Douglas on the anthropologist's concept of pollution; it might, even more appropriately, have quoted the traditional Middle English meaning of the word: "to render ceremonially or morally impure, desecrate, destroy the sanctity of. . .".

For this is what Professor Cotgrove is driving at: the idea that environmentalists are not out just to tidy up land, air and water (to do no more than that would be rejected as "cosmetic" treatment). They are out to overthrow the lifestyle that has, in their view, desecrated the countryside and de-humanized cities, and to put in its place what Illich calls "conviviality". A few years ago this sort of protest would have been academic in the pejorative meaning of the word. But a change has occurred that makes this protest potentially significant: it is the undeniable loss of confidence in the capacity of our political system to cope with our social and economic problems, a "decline in political legitimacy". To come back to Professor Cotgrove's statistics: it is not surprising that 74 per cent of environmentalists have little trust in political parties; it is surprising (at any rate, it ought to be) that 68 per cent of industrialists, too, have "little trust". And even more surprising that, when asked whether they support direct (extra-parliamentary) action to influence government decisions on environmental issues, there is still support from 23 per cent of the industrialists who responded to the questionnaire. The framework of consensus within which protest groups could

easily be tolerated is showing ominous signs of weakness. Professor Cotgrove suggests (as have, of course, many others before him) that the change from industrial to post-industrial society may not be a smooth one. Indeed, if he is right to use the word "paradigm" for the ruling lifestyle and the challenge being made to it, then the two paradigms cannot co-exist (that is why I don't think "paradigm" is the best word to use). And the question becomes not "whether?" but "how?" the transition will take place.

It is at this point, at the end of the book, that the reader may wish Professor Cotgrove had carried him a bit farther. We have to be content with I'd call the Heilbroner formula: get your house in order by constitutional means, or you'll find the constitution swept away and replaced by Leviathan. The urgent need is for reflection on the kind of political institutions which might evolve, from what we have to what we need. In his book *Ecology and the Politics of Scarcity* (which Professor Cotgrove does not refer to) William Ophuls has made some suggestions which could be the prolegomenon to an environmental ethic. Sociologists have an important part to play in finding out how such an ethic could be disseminated among a people still living (as Professor Cotgrove puts it) under a "Cornucopian faith". □

Eric Ashby is a Fellow of Clare College, Cambridge. He is joint author with Mary Anderson of The Politics of Clean Air, published by the Clarendon Press in 1981.

Keeping nuclear weapons inside the club

Stephen M. Meyer

Controlling the Bomb: Nuclear Proliferation in the 1980s. By Lewis A. Dunn. Pp.209. Hbk ISBN 0-300-02820-2; pbk ISBN 0-300-02821-0. (Yale University Press: 1982.) Hbk \$21, £16; pbk \$6.95, £5.45.

NINETEEN eighty two has been a fairly active year for watchers of international conflict. Britain and Argentina went to war over the Falkland Islands. The Iran-Iraq War flared to new levels of ferocity, culminating in the largest tank battles since the Second World War. Meanwhile, Israel engaged PLO and Syrian forces in Lebanon.

It is worth pondering how each of these conflicts might have evolved had they taken place in 1992, when many of the protagonists might have added nuclear weapons to their arsenals. What might have been the result of the detonation of an Argentine nuclear warhead in the midst of the British naval task force? What would have happened if the Iraqis, Iranians or both had nuclear bombs for their tactical

aircraft? And imagine the effects of sudden demonstration by the PLO that it had acquired a nuclear weapon (by theft, purchase or gift). In fact, none of these is implausible, and unless we wish to see such items in future news headlines we must begin to take a more serious and systematic approach to coping with the problem of nuclear weapons proliferation.

It is in this context that Lewis Dunn's *Controlling the Bomb* makes several timely contributions to our understanding of the spread of nuclear weapons, the threats posed by further proliferation and the options for slowing its pace and scope. In what is, perhaps, the most important aspect of this book, Dunn discards two pernicious notions currently much in vogue: that nuclear proliferation is essentially a technological phenomenon, and that further proliferation may have benign — if not beneficial — effects.

The first notion has dominated the literature since the beginning of the 1970s. Nuclear weapons proliferation was seen as a genuine technological imperative, a

deterministic outgrowth of the presence of nuclear power plants. Accordingly, the eradication of nuclear power technology (and associated plutonium stocks) was prescribed as the most effective approach to minimizing, if not eliminating, that threat. In reviewing events of the past decades, Dunn exposes the fundamental fallacy of this view. While technical difficulties have indeed been a factor in some cases of nuclear restraint, decisions to pursue — or abstain from — the acquisition of nuclear weapons have been driven by politico-military considerations. Efforts by Israel, South Africa, Libya, Iraq and Pakistan to acquire nuclear weapons cannot be explained by the corrupting influences of indigenous nuclear power generation because the former preceded the latter. Conversely, the failure of West Germany, Japan, Sweden, Switzerland, Canada, Belgium, South Korea and some two dozen other countries to produce nuclear weaponry cannot be accounted for by a lack of expertise. In either case, the total eradication of nuclear power technology would not affect these countries' long-term capability of putting together a nuclear weapons programme using indigenous resources.

As Dunn implies later, technical problems do play an important role in non-proliferation efforts simply by buying time. However, the effectiveness of technological constraints is undergoing continual erosion. Dunn points to the sources of trouble. In their rush to increase export revenues and markets, the primary supplier states have been backsliding on controls over sales of sensitive nuclear know-how and products. Grey and black marketeering have added to the flow of nuclear goods. Compounding the problem is the rise of third-tier nuclear suppliers — India and Argentina, for example. In the end the ability to produce nuclear weapons is inherent in the scientific, technological and industrial growth that is part of economic development.

While he does not see an inexorable drift towards the further spread of nuclear weapons, Dunn acknowledges that there are likely to be a number of instances where the confluence of technical expertise and political will produces new members of the nuclear club. Is there any reason to interfere, to try to alter the pace and scope of proliferation? Here Dunn confronts the second misconception. The presence of nuclear weapons, it is argued, can bring peace and stability to conflict-prone areas, such as the Middle East, by establishing regional deterrent systems similar to that which has prevented a US-USSR war for almost 40 years. Just as the porcupine can walk safely through the forest, so too can the small nuclear power dwell among a world of hostile countries. In explicitly rejecting such bizarre formulations, Dunn points to a host of factors that invalidate these analogies, while suggesting considerable threats ahead: the geo-

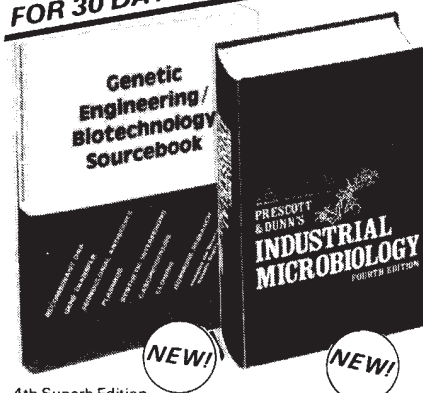
graphical proximity and political instability of those countries which may come to possess the bomb, superpower relations with such nations, and terrorist activities.

Recognizing the clear threat posed by the wider possession of nuclear weapons, the second half of *Controlling the Bomb* outlines a range of alternatives for a US anti-proliferation strategy. The first set of measures is aimed at checking the pace and scope of further proliferation. Dunn argues that — in order to buy time — technical barriers should be increased so as to make entry into the nuclear club as difficult as possible. Dunn notes that pilot-scale and research facilities can pose a greater threat of proliferation than full-scale commercial nuclear power facilities. Also, the United States should continue to limit the number of countries engaged independently in fuel reprocessing, while re-establishing itself as a reliable supplier of nuclear fuel. Multinational nuclear research and energy programmes (for instance to deal with international plutonium storage) should replace national projects, thereby giving developing countries access to advanced nuclear technology but without the risks associated with independent national programmes. Closing of technology export loopholes is necessary as well.

In order to affect relevant incentives and disincentives, Dunn also proposes a US strategy of political, economic, military and diplomatic sanctions. The objective is to impose such large drawbacks that they overwhelm any existing urge to acquire nuclear weapons. As Dunn emphasizes, this can only be accomplished through case-by-case analysis and direct intervention, not by some global or universal non-proliferation policy. In this respect, he notes — but, unfortunately, does not pursue in great detail — the fact that day-to-day US foreign policy does have an important effect on the balance of proliferation incentives and disincentives; for example the pursuit of other, frequently more important, foreign policy objectives may conflict with non-proliferation interests as often as they reinforce such interests.

A second set of measures is concerned with "limiting damage" in face of further proliferation. Dunn argues that there may be "natural" stopping points in the development of nuclear weapons — so-called proliferation firebreaks — behind which nations could be held by the threat of sanctions and other forms of diplomatic intervention. Where the drive towards the acquisition of a nuclear arsenal could not be prevented completely, emphasis could be placed on retarding the further evolution of the budding, but still small, nuclear force. Some countries might be contained at the convert (untested weapons) stage (e.g. Israel); some at the PNE (peaceful nuclear explosive) stage (e.g. India); some at the small stockpile stage; and so on.

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Lastly, the task of mitigating the consequences of nuclear proliferation is addressed. Dunn considers three thresholds to contend with: after nuclear weapons production is first detected; after a first nuclear test; and after nuclear use in warfare. An assortment of possible US responses is suggested. Technical assistance might be offered after the fact to reduce the dangers of unauthorized or accidental use. Security guarantees could be given to non-nuclear weapons states likely to be affected by the newly born nuclear power. Adjustments to alliance relationships may be required. And finally, consideration must be given to military intervention against a budding nuclear force that poses a clear threat to peace, or against one that comes under the control of non-governmental forces.

Dunn concedes that many of the policy

options in each of these three categories of control conflict with one another. As a result, the possibilities for developing a coherent US non-proliferation strategy are quite small. Thus, the reader should not be surprised to find no single blueprint for ensuring a world in which nuclear weapons are no more widely available than they now are. Controlling the bomb will require careful attention to decision-making in individual countries, and to the development of an approach to the problem that has the flexibility and versatility to tailor responses to the case at hand. Dunn deserves much credit for getting our thinking back on the right track. □

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Where are they now?

James S. Trefil

Contact with the Stars: The Search for Extraterrestrial Life. By Reinhard Breuer. Pp.292. ISBN 0-7167-1355-1. (W.H. Freeman: 1982.) \$28.50, £11.95.

IT IS HARD to criticize a man who agrees with you. In *Contact with the Stars*, newly translated from the 1978 German publication, Reinhard Breuer presents a view of extraterrestrial life which is becoming something of a new orthodoxy in science: the view that the development of life and of technology is not nearly as straightforward as was thought during the salad days of the 1960s, and that humanity, far from being a junior member of a widespread "galactic club", may, in fact, be a unique feature of our galaxy.

Such a view has many sources, one of which is the pioneering work of Michael Hart on the evolution of planetary atmospheres. Hart showed that the various forces that shape atmospheric development — chemistry, vulcanism, stellar evolution and the effects of life itself — comprise a finely balanced system that would require very little in the way of perturbation to upset it. In the case of the Earth, for example, the atmosphere has been undergoing changes in composition over its four-billion-year history, a period when the brightness of the Sun was increasing by 25 per cent. Life is possible on the Earth only because these two changes took place in exactly the right sequence to cancel each other out. One misstep and the Earth would have experienced a runaway greenhouse effect like Venus or become a frozen ball like Mars or Titan. As I write this, watching my daughters playing in the surf at Cape Hatteras, it is hard to imagine that everything I see — the clouds, the people, the ocean itself — is the result of a happy set of chance occurrences that are unlikely to have been repeated elsewhere. Yet that is precisely what the past 20 years of research on the origins of life tells us.

Another strong argument against the existence of copious numbers of extraterrestrial civilizations is attributed (perhaps apocryphally) to Enrico Fermi. When first presented with the statement that the number of such civilizations in the galaxy could well surpass a million, he is supposed to have remarked "Where is everybody?". The point is that it does not take much imagination to envisage the emergence of humanity as a star-faring race. The idea that we have the technical ability to colonize the entire galaxy is not difficult to accept in the age of space exploration, and calculations tell us that a wave of colonization centred on the Earth would sweep through the galaxy in some 30 million years — a mere blink of the eye in the lifetime of the cosmos. If there really are a million other races out there, most of them will be older and more advanced than

Bytes and pieces from an expert hand

Margaret A. Boden

Machine Intelligence and Related Topics: An Information Scientist's Weekend Book. By Donald Michie. Pp.316. ISBN 0-677-05560-9. (Gordon & Breach: 1982.) \$55.

DONALD Michie has played a crucial role in the development of artificial intelligence (AI) in Great Britain. He was a moving force behind the first British AI department, set up nearly 20 years ago in Edinburgh and recognized as of world-class stature. His commitment and enthusiasm derives partly from his friendship as a young man with Alan Turing (a colleague at Bletchley during the Second World War), on whose work our understanding of computation is grounded. The experience at Bletchley was influential too in its emphasis on the applications and social context of science, and on the value of interdisciplinary cooperation in scientific research.

Michie's intellectual pedigree commands attention, and not surprisingly there are many nuggets of interest in his book. But this collection of papers is, overall, disappointing. It is an uneasy mixture, ranging from ephemeral two-page journalistic pieces, through accounts of AI aimed at the general reader, to technical discussions of chess programs and probability theory. It includes personal memoirs of Turing, advice to eminent scientists in danger of being seduced into administration and circuit-lecturing, and impassioned criticism of the Science Research Council's Lighthill Report — which seriously hindered the progress of AI in Britain a decade ago and from which the field has not yet recovered.

The examples of machine intelligence on which Michie concentrates are expert

systems and chess programs. Expert systems are codifications of knowledge and inference about specific topics, such as geology or the diagnosis of infectious meningitis. The general interest of these is obvious, and his description of a variety of such knowledge-based systems (some of which are already in professional or commercial use) is lucid and informative. Chess programs might appear to be mere toys, but Michie argues that chess is especially well-suited to the experimental development of AI. It is a complex but relatively well-understood and clearly defined domain, and it demands research into techniques of heuristic search, pattern-recognition and inductive learning. AI work in other areas — such as language-understanding — is mentioned, but does not receive the detailed attention given to chess and expert systems.

Each paper in this collection was appropriate, even useful, in its original context. Michie is technically sophisticated, lucid in expression, historically sensitive and imaginative with respect to the future potential of AI. Few readers will find nothing of interest here. But it would be a strange person — even if he was that odd beast, an information-scientist — who would devote much of a weekend to reading this book. Despite Michie's advice that eminent scientists should spend their time doing innovative research rather than surveying their field, I hope he will one day give us what he is so well equipped to write: a sustained discussion of machine intelligence and its social context. □

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we are, so one can argue that the fact that they're not *here* is strong *prima facie* evidence that they're not *there*, either.

These two types of evidence — one from the study of the origin of life, the other from consideration of the human future in space — have convinced many members of the scientific community that the optimistic estimates of the abundance of extraterrestrial life bandied about in the 1960s and 1970s were simply wrong. As so often happens when new evidence arrives on the scene, old orthodoxies must give way to new. The "galactic club", conducive as it may be to good science fiction, is no exception to the rule.

Contact With the Stars must be understood, then, in the context of the general scientific counter-revolution on the subject of extraterrestrial civilizations. The new ideas have yet to percolate through to the general public, of course, but they are already well known to the experts. The book touches on all of the main topics related to the development of life — stellar evolution, genetic coding, space colonies, UFOs and so on. Some of the topics are treated very well. For example, I got a great deal out of Breuer's discussion of the debate on whether or not we have reached the end of the line in biological evolution. Unlike some who claim that a vastly superior human race will result from genetic engineering, Breuer argues that the genetic code is already carrying just about all the information it can, given the unavoidable copying errors which must occur in replication. This general attitude of informed scepticism is the greatest strength of the book.

On the debit side, the book reads more like a series of unconnected lectures than a single, cohesive document. The author often ventures into asides that range far from the topic under consideration. For example, after having repeatedly asserted his belief that we are alone in the galaxy, he launches into a lengthy and detailed discussion of strategies for communicating with the very extraterrestrials who aren't supposed to be listening. One can only wonder as to why so much effort has been spent devising complex pictographic codes if attempts to communicate are necessarily doomed to failure. □

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Books for Christmas

THE next review supplement to be published in *Nature* is Christmas Books, which will appear in the issue of December 9th.

As well as articles on recent science books for children, and on bird books and space science/astronomy books, the supplement will include reviews of *Aha! Gotcha*, *Darwin for Beginners*, *More Random Walks in Science*, *The Cult of the Expert* and *A Geological Miscellany*.

Time as time out of sight and mind

P.W. Atkins

On Time: An Investigation into Scientific Knowledge and Human Experience. By Michael Shallis. Pp.208. ISBN 0-09-148950-4. (Burnett Books/Hutchinson: 1982.) £8.95.

THERE are three types of scientist. One type believes that the whole of human experience is open to scientific investigation and rationalization. Another type believes that there are aspects of the world that lie outside the scope of science. The third type doesn't care one way or the other, and simply gets on with the job. The author of *On Time* is a member of Class 2; I am a member of Class 1, and therein lies a profound disagreement.

Dr Shallis has addressed himself to one of the most elusive aspects of the world: the nature of time. He takes us through a sequence of attitudes to time, ranging from its measurement, through its role in physics (encompassing relativity, entropy and causality), and ends with a series of chapters dealing with what most hard-nosed scientists regard as outside their domain, and lying beyond credulity. All this he treats with an engaging earnestness that fully captures his intention of approaching his subject with "the scientific attitude . . . of a wide-eyed child".

I suspect that you will like this book if you believe that there are aspects of the world outside science; for all *aficionados* of the paranormal warm to support from within the ranks of practising scientists. I suspect, also, that if you do not believe that science is incapable of dealing with these purported phenomena, such as by dismissing them as hoaxes or by explaining them in terms of established physics, then you will find this book a distressing mish-mash of credulity and passion. What you will not be able to complain about is, as the author so disingenuously reminds us, the impossibility of not going the whole hog on the paranormal once you have embarked on its foothills.

I was particularly struck by one phrase that occurs early on, where Dr Shallis remarks that, by extending conventional "instructional" science to encompass the unreproducible, occasional events that he thinks sometimes obtrude into the world too spasmodically to be captured by scientific method, he provides a description of reality which "will inevitably be richer than the former one". It seems to me that exactly the same remarks are made in favour of the use of hallucinogenic drugs; and with as much force. The heightened richness Dr Shallis asks us to accept (while acknowledging that it may inspire scorn) includes just about everything that may be regarded as symptomatic of crackpots: astrology, precognition, angels and gods (with, I think, fairies and bent teaspoons thrown in

for good measure). Some idea of the style of argument is captured by the manner in which the book speaks of α -emission. Apparently, this process is "absolutely causeless" (p.119) except for the possibility that it may be "influenced by mind" (p.125) — particularly, it seems, the minds of young chicks.

I hope that enough has been said to represent without distortion the thrust of Dr Shallis's argument: be totally credulous, swallow the whole of the paranormal, give weight to the hallucinations of the wishful thinking and the downright potty, and disregard the successful continuing progress of modern science. If you accept that argument then you will indeed accompany the author as far as his conclusion, that time is intrinsically paradoxical and must for ever remain elusive. The rejection of that argument, which in the face of the evidence presented here doesn't seem to present much difficulty, keeps open the path to comprehension. □

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Breaking the laws

Michael Berry

The Accidental Universe. By P.C.W. Davies. Pp.139. Hbk ISBN 0-521-24212-6; pbk ISBN 0-521-28692-1. (Cambridge University Press: 1982.) Hbk £10, \$19.95; pbk £4.95, \$9.95.

WHAT would the world be like if the force of gravity were a little stronger? If the proton were a little lighter? It is the purpose of Paul Davies's latest book to study questions such as these and, more generally, to explore the sensitivity of various features of the Universe to the values of the fundamental constants of nature. As groundwork the book begins with an account of the laws of physics and the scales on which they operate, from the shortest "Planck" lengths and times, through those characteristic of nuclear physics, up to cosmological ones.

The main body of the work is devoted to showing how these levels of structure are exquisitely interlocked, so that a change in one level can have astonishing repercussions elsewhere. For example, if the strength of the weak nuclear force (compared to gravity) were slightly different, this would greatly affect energy transport in stars, and probably prevent supernova explosions spewing forth the heavy elements which condense into planets and

ultimately ourselves. If the strong nuclear force were a few per cent stronger or weaker there would probably be no stars at all. And if the primordial energy density had been minutely different, the Universe would either have re-contracted implausibly long ago (and long before stars and planets could have formed) or exploded so violently as to inhibit condensation into galaxies. Related to this sensitivity are the celebrated "coincidences" involving large numbers; for example, the ratios of the age of the Universe to the time for light to cross a nucleus, and of the strength of gravity to the strength of electromagnetism, are both about 10^{40} .

By examining an impressive variety of such cases (basing his arguments largely on a review article by B.J. Carr and M.J. Rees — *Nature* 278, 605; 1979), Davies concludes that the Universe is so "fine-tuned" to its present condition by the values of fundamental constants that even a very small alteration would produce a world vastly different from ours and almost certainly unable to support life physically constituted as we know it. This leads him into a discussion of the anthropic principle, which in its weak form asserts: "What we can expect to observe must be restricted by the conditions necessary for our presence as observers", and in its strong form asserts: "The Universe must be such as to admit the creation of observers within it at some stage".

This sudden emergence into cosmology of the idea that the physical structure of the Universe is very special and related to its cognizability is rather strange. It seems to be connected in a curious way with the current preoccupation in applied mathematics with the concepts of genericity and structural stability as especially emphasized by René Thom. Much effort is being expended to determine the nature of the generic (that is, typical, or non-special) solutions of the equations representing physical laws, in the belief that these, being stable against perturbation, can represent persistent structures (condensed matter or life, for example). But the equations themselves are highly non-generic; Maxwell's laws of electromagnetism, for example, are a very special set of linear relations which strongly constrain two vector fields, and any modification would lead to wrong "laws". In this picture of generic solutions of non-generic equations it is difficult to see how the fundamental constants fit in: are they "random initial conditions" or are their ratios determined by laws we do not yet know?

The consequences of physical laws have been explored much more extensively employing the actual ratios of fundamental constants than with hypothetical modified values. It might be that evolution towards structural complexity is virtually irresistible, would survive such modification and still lead to intelligence (albeit with a physical basis quite different from ours — Olaf Stapledon's novel *Star Maker* comes

to mind, with intelligence developing via organized fluid motion in the wisps of galaxies and, later, inside stars). If this is correct — that is, if in the space of fundamental constants it could be shown to be generic for universes to have intelligence as an "attractor" of physical laws — the strong anthropic principle would follow as a marvellous consequence, and this aspect of the "coincidences" would lose its mystery.

Paul Davies does not devote much space

to such speculations but quite properly concentrates on explaining the physics underlying them. He does this with the lucidity and authority we have come to expect from him. The book should be accessible to anyone with an undergraduate acquaintance with physics, and I warmly recommend it as an excellent introduction to this new and important idea. □

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Common ground in a changing climate

Hubert Lamb

The Earth's Climate: Past and Future. By M.I. Budyko. Pp.307. ISBN 0-12-139460-3. (Academic: 1982.) \$39.50, £26.20.

THIS important book by the Soviet Union's outstanding climatologist, Professor M.I. Budyko, is a landmark in the development of climatology as a branch of physical science. At the same time, because climate itself touches more or less every aspect of life on Earth, and because of the way the author draws on the varied works of his long and active career, the book ends by being a statement of Budyko's life philosophy and perhaps of the broadest view of the world as seen from the Soviet Union. As such, it is not only an important work (and one written with notable lucidity) but of wide interest.

Since about 1960 Budyko has been actively involved in concern over man's impact on the environment and with the possibility that the overall effect of the accelerating increase of carbon dioxide in the atmosphere might so disturb the balance of in- and out-going radiation as to lead to an unacceptable warming of the Earth. He was also among the first to discuss the feasibility of counter-measures, such as introducing an aerosol veil into the stratosphere to reduce the warming.

The Earth's climate is some 40°C warmer than would be expected at this distance from the Sun thanks to the extent to which out-radiation is intercepted by the carbon dioxide, water vapour and so on in the atmosphere. Among the most interesting features of Budyko's book is the way in which he traces the changes of climate through the geological past in terms of the carbon dioxide and water supplied by volcanic activity. Decline set in as the volcanism eased off and as vegetation appeared and increased, removing carbon dioxide and converting it to oxygen. This decline was accompanied by a lowering of temperature and the appearance of polar ice. Other shorter-term temperature drops have been associated with periods of specially active volcanism and frequent loading of the atmosphere with veils of dust and aerosol.

However, not all geologists agree that the record of carbon dioxide in the atmosphere fits so well with the known history of prevailing temperatures. Nor can the temperature history of the past century or more be explained by carbon dioxide alone. I am concerned that not only Budyko's book but prevailing opinion among leading meteorologists and climatologists may seriously over-emphasize the importance of carbon dioxide *vis-à-vis* other causes of climatic change. In this book it turns out that in 101 pages carbon dioxide is considered as a major cause of climatic changes, compared with 21 pages for volcanism, and 25 pages for the capacity of polar ice variations and other albedo variations to amplify the overall effect on world temperature. Possible variations of the solar constant get two pages, variations of cloudiness five pages, and continental drift/polar wandering and the Earth's orbital variations about four pages each. The last named variables are surely under-stressed.

The frequent forecasts nowadays, in this book and elsewhere, of a coming drastic change of climate due to man's production of carbon dioxide (and other effluents) are in general a good deal too confident. Despite its basis in the laws of physics, so well expounded in this book, climate is the product of a complex of influences that defies precise modelling. There is no clearly definable limit to the range or the rate of natural climatic variability, particularly the coolings associated with waves of volcanism and (perhaps) other causes. Once within the past 300 years, between 1690 and 1750, there was an oscillation which changed the ten-year average temperatures in England by nearly 2°C, probably the local expression of a global phenomenon. Carbon dioxide changes can be presumed to have played no part in this. The prevailing confidence in the carbon dioxide forecasts is based on their grounding in straightforward physics, but our experience of the difficulties of short-range meteorological forecasts is not entirely irrelevant to this problem.

That said, the possibility of a drastic

climatic warming due to the carbon dioxide has nonetheless to be taken seriously and Budyko has long been pressing upon scientists and politicians alike the urgency of the matter. On the one side, the increase of carbon dioxide could, if its effect were not counteracted by natural or anthropogenic influences, warm world climate by as much as 3°C within about 50 years — and possibly by 10–15°C in the highest latitudes, with disappearance of the polar ice. This and the corresponding changes in the rainfall pattern would be expected to shift the agricultural crop zones and the desert boundary northwards by a highly inconvenient number of degrees of latitude. This would also initiate the rapid melting of the land-based ice-sheets in Greenland and elsewhere, possibly including the great ice-sheet in Antarctica, with a consequent rise of ocean level that would overwhelm the Netherlands and drown large parts of the world's biggest cities. On the other side, forward calculation of the astronomical (Earth's orbital) variations points to a new ice age beginning within about 5,000 years (not the 10,000 to 15,000 years mentioned in the book on the basis of an outdated calculation).

Against this background Budyko sees the increase in carbon dioxide through burning fossil fuel as a benign effect if only it can be moderated to a slower rate of change to which we may be able to adapt. In his eyes man is restoring the atmosphere to its state before the growth of vegetation and is thereby prolonging the possibility of existence of human life and of a biological environment sufficient for our needs. Otherwise the continuing natural decline which set in before the Pliocene would, he suspects, doom the biosphere to extinction within one million years. There are interesting asides in his final chapter about the uniqueness of the Earth's biosphere and the improbability of life, let alone technologically proficient beings, elsewhere in the Universe. On another page, he develops a vision of the future made possible by our postponement of the run-down of carbon dioxide in the atmosphere: a future with a much denser human population and more varied life-forms and, presumably, accelerated through-put of the food chains. Not everyone, however, will see that as progress to a better world.

But in the end there is a noteworthy agreement between the conclusions reached on both sides of the cultural and philosophical divide about our situation in the world today, particularly as regards the threat that man's activities present to our planet. Budyko quotes a Soviet colleague, mentioning a well-known statement by Karl Marx, that

culture leaves behind itself a desert if it develops spontaneously and is not directed deliberately ... a developed society ruins nature and destroys the environment from which it derives all its resources.

In this we are no better than our primitive ancestors, whatever their

thoughts about the world, who ran so many species of the animals they hunted to extinction. To avert the danger, mankind "must form [take charge of] nature on the global scale". This demand for a cultural dictatorship corresponds to the recognition in informed Western circles of our responsibility towards our successors on Earth and of the need for controls and restraints based on wise counsels and better knowledge. □

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Philosophy in retreat

Stephen Hawking

Cosmology, Physics, and Philosophy. By Benjamin Gal-Or. Pp.522. ISBN 0-387-90581-2. (Springer-Verlag: 1982.) DM 69.

THE ancients considered the ultimate question of "Life, the Universe, and Everything" to be part of philosophy even though they were not sure that the answer was 42. For instance, Aristotle wrote a book on the structure of the heavens in which he put forward good reasons for believing that the Earth was spherical and even gave a figure for the circumference that was correct to within a factor of two. Other philosophers up to and including Kant also considered the structure of the physical Universe to be within the arena of philosophy.

In more recent times, however, philosophers have largely abandoned cosmological questions to physicists and have shrunk the scope of their enquiries to such an extent that many of them now hold that "the sole remaining task for philosophy is the critique of language". Although physicists have brought about great changes in our ideas about the Universe and in our concepts of space and time, they tend to shy away from questions that they regard as philosophical or metaphysical like "does time have a beginning" or "why is the Universe the way it is".

Benjamin Gal-Or, the author of *Cosmology, Physics, and Philosophy*, bemoans the gulf that has grown up between philosophy and cosmology and attempts to bridge it by informing philosophers about the modern theories of physics and by trying to interest physicists in philosophy. In the first of these endeavours he may have some success. The book contains a reasonable account of the hot big-bang theory. It is however overlaid with a lot of other ideas from the modern literature on theoretical physics which the author has included rather uncritically, even though they may not be relevant to the main contents or they may be ephemeral

and not taken very seriously by others in the field. An example of this is a flow diagram in the introduction which is meant to show the relations between the main ideas in the book but which is more like a maze with about 40 or 50 boxes connected by arrows. I feel that non-physicists who want to find out about modern theories of the Universe might do better to read the clear and reasonably short book by Steven Weinberg entitled *The First Three Minutes*.

The book's attempt to inform physicists about philosophy is not very successful, at least as far as this physicist is concerned. I found it rather confused and verging on the mystical. One is frequently enjoined to cast aside prejudices and to embrace "gravitism" and "havayism", which is supposed to be the science of the whole, though it was never clear to me what one was supposed to do with them. I also found it rather irritating to have about a quarter of each page in italics for emphasis. Nevertheless, the task that Gal-Or has attempted is a very important one and this book will have to serve until someone writes a better one. □

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In muddled waters

Michael Ruse

The Shaping of Man: Philosophical Aspects of Sociobiology. By Roger Trigg. Pp.186. Hbk ISBN 0-631-13023-3; pbk ISBN 0-631-13028-4. (Basil Blackwell: 1982.) Hbk £12.50; pbk £5.95. To be published in the USA next year by Schocken Books.

THE study of animal social behaviour from an evolutionary perspective dates from the publication of Darwin's *Origin*. But, for a number of reasons — not the least of which is its sheer difficulty — such study languished for a century; and this despite the valiant efforts of a number of workers, most notably and creditably the so-called "ethologists". However, in the past 10–15 years, this member of the family of evolutionary science has awoken, and now races ahead, both theoretically and empirically. Under the new rubric of "sociobiology", the biological basis of animal social behaviour is a rich hunting ground for the brightest and most ambitious of graduate students.

Yet, not everyone has welcomed sociobiology; at least, not everyone has welcomed sociobiology as applied to our own species, *Homo sapiens*. Expectedly, left-wing thinkers are wary of any attempt to relate human behaviour to genes, and they have been joined in their opposition

by others. To my regret, members of my own discipline, philosophy, have added their voices to the chorus of dissent, happily "proving" that not only is human sociobiology pernicious and dangerous, but that it is also conceptually impossible! I speak of "regret", because although it is undoubtedly the case that some pretty daft things have been said by advocates of human sociobiology, it surely should be of some moment to us as philosophers that human beings are modified monkeys, rather than the miraculous recent creations of a Being determined to produce offspring in His own image.

The pendulum of opinion now appears to be swinging back, however, and Roger Trigg's new book is happy confirmation of this fact. He is far from an uncritical devotee of human sociobiology, but equally he is convinced that as philosophers — as inquirers into the ultimate nature of man — "we reject the findings of human biology at our peril" (p. xviii).

What is man? Trigg runs through a number of answers and this large survey is one of the irritating aspects of the book; it takes an interminable time before the author turns to his sub-title and to biology. For some one hundred pages we get a Cook's tour, as Trigg takes us rapidly through a selection of the many non-biological answers that have been given to his question. In fairness, the tour is not without value. I am reminded of Woody Allen's comment about how philosophy frustrated him, because he could never tell the difference between heuristics and hermeneutics. He should read Trigg's book, for he will find the solution to that problem, as well as to many others.

Specifically, Trigg introduces us to those such as B.F. Skinner and David Hume who would treat man as object. Some familiar, but nonetheless pertinent objections are raised, for instance the inability of those who regard man as an object to regard themselves as objects! Then Trigg looks at those who take the opposite tack, regarding man as subject. Included here are such thinkers as J-P. Sartre, R.G. Collingwood and H. Gadamer. The difficulties in this approach are noted, including that of ever escaping from the relativism which stems from the overall presuppositions. Next, Trigg looks at sociological approaches to man (his answer is that they are limited), and discusses whether man is a genuine species (yes, but this doesn't tell us as much as we might hope).

And so, finally, we get to the question promised by the book's title: "Is biology the key to the nature of man?". Trigg's answer is guarded. On the one hand, he agrees that biology — specifically human sociobiology — seems to have at least the potential to throw light on human behaviour and its causes; on the other, he warns of the limitations and confusions in the biological approach.

I confess that there were times through this part of his discussion when I felt that Trigg was being a little less than fair to sociobiology (or, at least, to its proponents). Sociobiologists are certainly aware of obvious traps. For instance, if one explains behaviour through (say) kin selection, then although causally one is thinking in terms of reproductive benefits to the individual, it does not follow that one can draw sweeping claims about all humans being "selfish" or some such thing. Talk of "selfish genes", for instance, is metaphorical, and does not commit sociobiologists to the thesis that every human being is a conscious self-interested schemer.

Yet at this point, Trigg accuses sociobiologists of "muddying the waters" for they juxtapose "altruism with an ordinary notion of advantage and self-interest" (p.117). Rather it is Trigg who is muddying waters, or at least who is accusing sociobiologists of being more naive than they really are. Surely he would not suggest that any attempt to give a causal analysis of human behaviour is doomed to failure? Nor even would he suggest that all causes must be related at once to our immediate conscious awareness. Suppose one says that an adult behaves well because of strict childhood discipline. This may or may not be true, but seems to make sense if the adult does not usually consciously remember the childhood training. If this is so, why is it wrong in principle for sociobiologists to formulate explanations using factors which operate below (or outside) the level of consciousness? And why should they not use metaphors such as "selfish"? Every other scientist uses metaphors (force, work, energy, power, attraction, repulsion and so on).

Finally, Trigg raises the relationship between biology and morality. Again, his conclusion is cautious:

The insights of sociobiology may give us further understanding of man's nature and give us material on which to base our claims. They cannot take away from us the capability of rational moral choice, based on our conception of the nature of man. The paradox is that any attempt through rational argument to treat man as merely another animal, no different from other animals, is itself self-refuting [p.162].

I'm not quite sure of the strength of this supposed paradox. Is it empirical, in that man simply seems different from other animals? Or is it necessary, in that the very attempt to explain ourselves proves that biology is limited in power? Either way, my own inclination is to agree with Trigg that there is more to morality than biology, in the sense that one certainly cannot deduce morality from biology. Our biological urges are not always "good" urges. Nevertheless, I would go further than Trigg — if indeed we are the product of evolution, then our moral sense presumably has some adaptive value, and hence biology can perhaps throw light on

the content of our biology. Biology may not justify in some absolute way our sense of the worth of human being, but it can show why we have such a sense. And perhaps this is all the proof we can ever hope for.

Clearly I don't agree with some of Trigg's conclusions, any more than he would with mine. But that is the nature of philosophy (nor is such disagreement a sign of any inherent weakness in the subject). What counts is that although he is over-long in getting to his main theme, Trigg stimulates one to think on important matters. And that is no small praise. □

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Good, bad & biology

Marian Dawkins

The Nature of the Beast: Are Animals Moral? By Stephen R.L. Clark. Pp.127. ISBN 0-19-219130-6. (Oxford University Press: 1982.) £7.95, \$14.95.

STEPHEN Clark has made a tactical error. He is a philosopher writing about animal behaviour and attempting to explore the possible analogy between what animals do and human morality. But he unfortunately fails to conceal his contempt for biologists and many of their theories about animals. "The philosopher at a gathering of biologists", he remarks in the Introduction, "is the one in a state of permanent exasperation that these scientists do not understand their own concepts!". He dismisses various biological theories as "merely ridiculous" or "broken-backed and to point this out should be a job for any competent editor, let alone a philosopher".

Writing about a subject which is not your own is always hazardous; but to write in such a way that you raise the hackles of those working in the field before you have even got down to saying what you want to say is hardly the way to persuade biologists that a dose of philosophy would be a good thing. I say this because my own reaction to the book was strongly coloured by resentment at a number of implications, such as that all ethologists are right-wing ideologues who have to be taught a lesson on how to think straight. This resentment probably made me less tolerant of factual errors, more irritated by grammatical awkwardness and far less open to the contribution this book may make than I would have been had exactly the same ideas been put over in a different spirit. For the subject of the book — whether what human beings regard as "moral" has anything to do with what animals have been shaped by

natural selection to do — is an important one. There has indeed been a lot of muddled thinking and perhaps facile generalizations from other species to our own. So having expressed my irritation, I will now try to concentrate on content rather than style.

Dr Clark is concerned with what he calls the "morals of Nature". Parent animals care for their children, do not always kill their rivals and apparently respect sexual taboos and figures of authority. There are at least superficial similarities between these examples and conduct which we would call moral if it were done by a human being. But would we be right to describe such behaviour as moral in a non-human species? Are the inhibitions that a wolf feels when it stops short of killing a rival anything like moral ones? And can we learn anything about human morality from studying animals?

To answer these questions, Dr Clark examines various aspects of animal behaviour, including parental behaviour, territory and dominance, drawing on considerable factual detail in the process. He then comes to the conclusions that other animals are not moral in the usual human sense of the word because they do not have the ability to see that they could be wrong. He describes animals as ethical because they "respond to aspects of a situation and to features of their kindred, that a good man would also respect". But he says they are not moral,

for they do not, as far as we can see, have any occasion to moralise about themselves or to construct intellectual systems to accommodate their immediate responses.

So far Dr Clark's conclusions are easy enough to follow (apart from reservations about some animals which behave in ways which a good man might not respect), but they are neither very surprising nor very different from common sense. It is when he tackles the question of whether human beings should, in any way, adopt the "morals of Nature" that it becomes much more difficult to see what he means. He believes that "the ways of the beasts can set us good examples". Yet he has already argued that the "beasts" are not moral, so it is not clear why he thinks they may set us moral examples. He also believes that some of our moral habits, such as caring for relatives, are "bred into" us. And yet he firmly states that morality does not and cannot come from biology.

One is, in short, left more confused at the end of the book than at the beginning. His scathing criticisms of biologists might have been a great deal easier to stomach if he himself had been able to show that clarity of thought that he feels biologists so obviously lack. □

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Separated learning and symbol making

Carolyn A. Ristau

The Unique Animal: The Origin, Nature and Consequences of Human Intelligence. By Don D. Davis. Pp.336. Hbk ISBN 0-907152-02-3; pbk ISBN 0-907152-01-5. (Prytaneum Press: 1981.) Hbk £12.95, \$25; pbk £6.95, \$12.95.

DON DAVIS has a provocatively simple hypothesis of the difference between the minds of man and beast. He proposes that man alone is capable of separated learning — of proposing a connection between two or more events separated in time. This entails more than remembering over a long period of time; connecting two seemingly distinct events is essential. As a consequence only human beings can create symbols or discover a relationship between two non-contiguous events, and can hypothesize or propose such connections. Man's hypotheses flower in his magic, religion and science. Man alone has placed a seed in the ground one day, seen a plant grow at that place days later and connected the two events.

But is this the domain only of human beings as Davis claims? Ethologists might note, for example, that certain termites grow fungi and that some ants capture, tend and "raise" aphids for milking, although no one has shown that an individual insect could "discover" the relationships involved. Again, whether man is the only symbol maker seems highly debatable. At least some apes seem to have learned symbols taught by man — as Davis agrees — and some apes' use of novel sign combinations may be indicative of an ability to create a symbol. To conclude that natural animal communication is not symbolic may merely reflect a limitation of our methods of study. For example, the honey bee's dance may be a symbolic

achievement when it is used to indicate the location of food.

Equally relevant is the question of whether man is the only creature that in learning can tolerate gaps between environmental events of more than a few seconds. Davis dismisses the obvious counter-examples — taste aversion learning and events that are marked by an especially salient stimulus such as a loud noise — as extremely narrow exceptions. Yet whether animals, like human beings, can "mark" events or locations (an ability that shares characteristics with the process of naming) remains a matter for both philosophical discussion and experimentation. Scent-marking by animals and food-caching with later retrieval may represent rudimentary forms of marking.

To be sure Davis's concept of separated learning is powerful, particularly given the way that contiguity as a prerequisite for learning has dominated experimental psychology for decades. No clear examples of such learning by animals come to mind, in contrast with the ease with which Davis is able to apply the concept to pre-historical science, the discovery of fire, of death's inevitability, of smelting, pottery, writing and record-keeping, and the interrelations of magic, religion and science.

Davis's book is of intellectual interest to a broad audience both scientific and popular. There are gaps in his references, however, particularly of pertinent recent research, leading to some incorrect generalizations; and some of his interpretations of research are questionable. But his ideas are important and provocative. They clearly merit attention. □

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The pitfalls of bookmaking (continued from p.112)

► space as an entry in a telephone book. The main thrust (as publishers like to say) is by direct mail which ideally means a leaflet, brochure or booklet landing on the mat of potential customers quickly and reliably. However postal and handling expenses have escalated in recent years and even publishers who specialize in this type of marketing have drawn in their horns somewhat. There can be months of delay before the information about a particular book, especially if it appeals to small groups in different disciplines, reaches all possible readers. Campbell is bound to be on the list which is mailed last.

In the first year of publication perhaps as many as 800 copies will have been sold even though no one seems to know about the book. A further 200 sales will appear on the statement for the following year and then probably no more than another 100 over

the next three years. Campbell, a reflective character, may wonder whether this is *the* way to reach the 1,100 purchasers who alone seem to be interested. Where is the electronic revolution? Even publishers stop and wonder, as they see the latest list of overstocks to be "wasted" because they are unsaleable, and ponder whether the print runs should in future be set below 1,000.

Meanwhile, over lunch with Parsons an adviser to the firm suggests there is room for a nice, tightly edited volume on the topic of Campbell's original chapter. The field has grown. The ideal editor would be Campbell himself, and he is approached. Does he accept? □

Anthony Watkinson, formerly Editorial Director of Academic Press, London, is now at Oxford University Press.

BOOKS RECEIVED

Astronomy

- CORADINI, A. and FULCHIGNONI, M. (eds). *The Comparative Study of the Planets. Proceedings of the NATO Advanced Study Institute held September 1981, Italy*. Pp.516. ISBN 90-277-1406-1. (Reidel: 1982.) Np.
- HOSKIN, M. *Stellar Astronomy: Historical Studies*. Pp.197. ISBN 0-905193-04-0. (Science History Publications: 1982.) £9.95.
- LEARNER, R. *Astronomy Through the Telescope*. Pp.224. ISBN 0-237-45644-3. (Evans Brothers, London: 1982.) £12.50.
- MARIOLOPOULOS, E.G. and THEOCARIS, P.S. (eds). *Compendium in Astronomy*. Pp.464. ISBN 90-277-1373-1. (Reidel: 1982.) Np.

Physics

- BASOV, N.G. (ed.). *Materials and Apparatus in Quantum Radiophysics*, Vol. 98. *Proceedings (Trudy) of the P. N. Lebedev Physics Institute*. Pp.162. ISBN 0-306-10964-6. (Consultants Bureau, New York: 1982.) \$39.50.
- BRODIE, I. and MURAY, J.J. *The Physics of Microfabrication*. Pp.503. ISBN 0-306-40863-5. (Plenum: 1982.) \$49.50.
- DESLOGE, E.A. *Classical Mechanics*, Vol. 1. Pp.459 + Appendix. ISBN 0-471-09144-8. (Wiley: 1982.) Np.
- DESLOGE, E.A. *Classical Mechanics*, Vol. 2. Pp.980 + Appendix. ISBN 0-471-09145-6. (Wiley: 1982.) Np.
- FENN, J.B. *Engines, Energy, and Entropy: A Thermodynamics Primer*. Pp.293. Hbk ISBN 0-7167-1281-4; pbk ISBN 0-7167-1282-2. (Freeman: 1982.) Hbk £13.50; pbk £6.95.
- FERRARO, J.R. and BASILE, L.J. (eds). *Fourier Transform Infrared Spectroscopy: Techniques Using Fourier Transform Interferometry*, Vol. 3. Pp.215. ISBN 0-12-254103-0. (Academic: 1982.) \$29.
- HARTNETT, J.P. and IRVINE, T.F. Jr. (eds). *Advances in Heat Transfer*, Vol. 15. Pp.352. (Academic: 1982.) \$54.
- LAWDEN, D.F. *An Introduction to Tensor Calculus, Relativity and Cosmology*, 3rd Edn. Pp.205. Hbk ISBN 0-471-10082-X; pbk ISBN 0-471-10096-X. (Wiley: 1982.) Hbk £14.75, \$30.50; pbk £6.75, \$14.95.
- THORPE, M.F. (ed.). *Excitations in Disordered Systems*. Pp.704. ISBN 0-306-40981-X. (Plenum: 1982.) \$85.
- TROWER, W.P. and BELLINI, G. (eds). *Physics in Collision: High-Energy ee/pp Interactions*, Vol. 1. Pp.521. (Plenum: 1982.) \$65.
- YIH, C-S. (ed.). *Advances in Applied Mechanics*, Vol. 22. Pp.327. ISBN 0-12-002022-X. (Academic: 1982.) \$53.
- ZICHICHI, A. (ed.). *Pointlike Structures Inside and Outside Hadrons*. Pp.739. ISBN 0-306-40568-7. (Plenum: 1982.) \$85.

Chemistry

- ALKEMADE, C. Th. J. *et al.* *Metal Vapours in Flames*. Pp.1033. ISBN 0-08-018061-2. (Pergamon: 1982.) £50, \$95.
- BERNSTEIN, R.B. *Chemical Dynamics via Molecular Beam and Laser Techniques: The Hindshelwood Lectures*, Oxford, 1980. Pp.262. Hbk ISBN 0-19-855154-1; pbk ISBN 0-19-855169-X. (Oxford University Press: 1982.) Np.
- COTTON, F.A. and WALTON, R.A. *Multiple Bonds Between Metal Atoms*. Pp.466. ISBN 0-471-04686-8. (Wiley: 1982.) Np.
- CROMPTON, T.R. *Gas Chromatography of Organometallic Compounds*. Pp.589. ISBN 0-306-40987-9. (Plenum: 1982.) \$75.
- DREYFUSS, P. *Poly (tetrahydrofuran). Polymer Monographs*, Vol. 8. Pp.306. ISBN 0677-03330-3. (Gordon & Breach: 1982.) \$59.50.
- HERCULES, D.M. *et al.* (eds). *Contemporary Topics in Analytical and Clinical Chemistry*, Vol. 4. Pp.381. ISBN 0-306-40943-7. (Plenum: 1982.) Np.
- KATRITZKY, A.R. (ed.). *Advances in Heterocyclic Chemistry*, Vol. 30. Pp.408. ISBN 0-12-020630-7. (Academic: 1982.) \$76.50.
- MACKIE, R.K. and SMITH, D.M. *Guidebook to Organic Synthesis*. Pp.338. ISBN 0-582-45592-8. (Longman: 1982.) £9.95.
- MINCZEWSKI, J., CHWASTOWSKA, J. and DYBCZYŃSKI, R. *Separation and Preconcentration Methods in Inorganic Trace Analysis*. Pp.543. ISBN 0-85312-165-6/0-470-27169-8. (Ellis Horwood/Halsted Press: 1982.) £37.50, \$63.50.

Computer Science

- GREEN, P.E. Jr. (ed.). *Computer Network Architectures and Protocols*. Pp.718. ISBN 0-306-40788-4. (Plenum: 1982.) \$59.50.
- HARE, V.C. Jr. *Basic Programming*, 2nd Edn. Pp.407. ISBN 0-15-505002-8. (Harcourt Brace Jovanovich: 1982.) £11.
- MEYER, R.E. (ed.). *Transonic, Shock, and Multidimensional Flows: Advances in Scientific Computing*. Pp.345. ISBN 0-12-493280-0. (Academic: 1982.) \$21.
- MITTLEMAN, D. *Basic Computing*. Pp.430. ISBN 0-15-504910-0. (Harcourt Brace Jovanovich: 1982.) £10.95.

Earth Sciences

- CLOWES, A. and COMFORT, P. *Process and Landform: An Outline of Contemporary Geomorphology. Conceptual Frameworks in Geography*. Pp.289. ISBN 0-05-003127-9. (Oliver & Boyd, Edinburgh: 1982.) £4.95.
- CONWAY MORRIS, S. (ed.). *Atlas of the Burgess Shale*. No ISBN. (The Paleontological Association: 1982.) £20, \$44 (for members £12.50, \$27.50).
- NEIBURGER, M., EDINGER, J.G. and BONNER, W.D. *Understanding Our Atmospheric Environment*, 2nd Edn. Pp.453. ISBN 0-7167-1348-9. (Freeman: 1982.) £13.95.
- PRESS, F. and SIEVER, R. *Earth*, 3rd Edn. Pp.613. Hbk ISBN 0-7167-1362-4; pbk ISBN 0-7167-1382-9. (Freeman: 1982.) Hbk £18.50; pbk £9.95.

- SCIENCE PRESS. *Geological and Ecological Studies of Qinghai-Xizang Plateau*. Vol. 2. of 2 Vols. Pp.2138. ISBN (Vol. 1.) 0-677-60210-3; (Vol. 2.) 0-677-60220-3; (2 Vol. set) 0-677-60230-8. (Gordon & Breach: 1981.) Vol. 1. \$150; Vol. 2. \$175; 2 Vol. Set \$290.

- TANKARD, A.J. *et al.* *Crustal Evolution of Southern Africa: 3.8 Billion Years of Earth History*. Pp.523. ISBN 3-540-90608-8/0-387-90608-8. (Springer-Verlag: 1982.) DM 118, \$55.

Biological Sciences

- ABELES, M. *Local Cortical Circuits: An Electrophysiological Study*. Pp.102. ISBN 3-540-1134-8/0-387-11034-8. (Springer-Verlag: 1982.) DM 39, \$18.20.
- BACONSFIELD, R. and BIRDWOOD, G. (eds). *Placenta: The Largest Human Biopsy*. Pp.160. ISBN 0-08-028028-5. (Pergamon: 1982.) £20, \$40.
- BAJEMA, C.J. (ed.). *Artificial Selection and the Development of Evolutionary Theory. Benchmark Papers in Systematic and Evolutionary Biology*, 4. Pp.361. 0-87933-369-3. (Academic: 1982.) \$47.
- BATE, R.H., ROBINSON, E. and SHEPPARD, L.M. (eds). *Fossil and Recent Ostracods*. Pp.492. ISBN 0-85312-324-1/0-470-27314-3. (Ellis Horwood/Halsted: 1982.) £38.50, \$84.50.
- BRECCIA, A. RIMONDI, C. and ADAMS, G.E. (eds). *Advanced Topics on Radiosensitizers of Hypoxic Cells*. Pp.284. ISBN 0-306-40915-1. (Plenum: 1982.) \$39.50.
- BREIPOHL, W. (ed.). *Olfaction and Endocrine Regulation. Proceedings of the 4th Chemoreception Research Organization Symposium and the 2nd International Laboratory Workshop held October 1981, Schloss Lembeck, FRG*. Pp.409. Pbk ISBN 0-904147-35-5. (IRL Press: 1982.) £13, \$26.
- BRINKHURST, R.O. *British and Other Marine and Estuarine Oligochaetes. Synopses of the British Fauna No. 21*. Pp.127. Hbk ISBN 0-521-24258-4; pbk ISBN 0-521-28559-3. (Cambridge University Press: 1982.) Hbk £15.50; pbk £6.50.
- BRITTON, D. and HAYASHIDA, T. *The Japanese Crane: Bird of Happiness*. Pp.63. ISBN 0-87011-484-0. (Prentice-Hall: 1982.) £9.30.
- BROKAW, C.J. and VERDUGO, P. (eds). *Mechanism and Control of Ciliary Movement. International Meeting held September 1981, Friday Harbor, Washington. Progress in Clinical and Biological Research*, Vol. 80. Pp.264. ISBN 0-8451-0080-7. (Alan R. Liss: 1982.) £30, DM 132.
- CHIARELLI, A.B. and CORRUCINI, R.S. (eds). *Advanced Views in Primate Biology. Main Lectures of the 8th Congress of the International Primatological Society held July 1980, Florence*. Pp.266. ISBN 3-540-11092-5/0-387-11092-5. (Springer-Verlag: 1982.) DM 98, \$43.60.
- CURDS, C.R. *British and Other Freshwater Ciliated Protozoa. Part 1, Ciliophora: Kinetofragminophora. Synopses of the British Fauna, No. 22*. Pp.387. Hbk ISBN 0-521-24257-6; pbk ISBN 0-521-28558-5. (Cambridge University Press: 1982.) Hbk £27.50; pbk £11.95.
- CUTLER, D.F., ALVIN, K.L. and PRICE, C.E. (eds). *The Plant Cuticle. Papers Presented at an International Symposium held September 1980, London. Linnean Society Symposium Series, No.10*. Pp.462. ISBN 0-12-199920-3. (Academic: 1982.) £48.40, \$99.50.
- DAVIS, E. (ed.). *Raynaud Features, Acrocyanosis, Cryoimmunoproteins. Advances in Microcirculation*, Vol. 10. Pp.116. ISBN 3-8055-2790-X. (Karger: 1982.) SwFr. 98, DM 117, \$58.75.
- DE BAIACLI LEVY, J. *The Illustrated Herbal Handbook*. Pp.224. Pbk ISBN 0-571-11894-1. (Faber & Faber: 1982.) Pbk £3.50.
- EASTERBY, R., KROEMER, K.H.E. and CHAFFIN, D.B. (eds). *Anthropometry and Biomechanics: Theory and Application*. Pp.327. ISBN 0-306-40745-0. (Plenum: 1982.) \$42.50.
- FRASER, J.H. *British Pelagic Tunicates: Keys and Notes for the Identification of the Species. Synopses of the British Fauna, No. 20*. Pp.57. Pbk ISBN 0-521-28367-1. (Cambridge University Press: 1982.) £4.95.
- FRTTZ, H., DIETZE, G. *et al.* (eds). *Recent Progress on Kinins. Agents and Action Supplements*, Vol. 9. International Conference held November 1981, Munich. Pp.708. ISBN 3-7643-1324-2. (Birkhäuser-Verlag: 1982.) SwFr. 118.
- GANTEN, D. and PFAFF, D. (eds). *Sleep: Clinical and Experimental Aspects. Current Topics in Neuroendocrinology*, Vol. 1. Pp.129. ISBN 3-540-11125-5/0-387-11125-5. (Springer-Verlag: 1982.) DM 78, \$34.70.
- GARFIELD, E. *ISI Atlas of Science: Biochemistry and Molecular Biology 1978/80*. Pp.540. ISBN 0-941708-00-4. (Institute for Scientific Information: 1982.) \$45, \$90 (institutions).
- GARRE, W.J. *The Missing Link: The Transition from Animal Instinct to the Human Mind*. Pp.293. ISBN 0-8022-2399-0. (Philosophical Library, New York: 1982.) \$15.
- GAWTHORNE, J.M. *et al.* (eds). *Trace Element Metabolism in Man and Animals. Proceedings of the 4th International Symposium held May 1981, Western Australia*. Pp.715. ISBN 3-540-11058-5/0-387-11058-5. (Springer-Verlag: 1982.) DM 149, \$69.40.
- GOODWIN, D. *Estrildid Finches of the World*. Pp.328. ISBN 0-19-858506-3/0-565-00832-3. (Oxford University Press/British Museum: 1982.) £25.
- GOOSE, D.H. and APPLETON, J. *Human Dentofacial Growth*. Pp.228. Hbk ISBN 0-08-026394-1; pbk ISBN 0-08-026393-3. (Pergamon: 1982.) Hbk £20, \$40; pbk £8.95, \$17.95.
- GRICE, G.D. and REEVE, M.R. (eds). *Marine Mesocosms: Biological and Chemical Research in Experimental Ecosystems*. Pp.430. ISBN 3-540-90579-0/0-387-90579-0. (Springer-Verlag: 1982.) DM 108, \$50.30.
- HICKMAN, C. P. Jr. *et al.* *Biology of Animals*, 3rd Edn. Pp.646. ISBN 0-8016-2167-4. (Year Book Mosby, London: 1982.) £17.75.

ARTICLES

Geochemistry of a Pliocene–Pleistocene oceanic-arc plutonic complex, Guadalcanal

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The Koloula Igneous Complex, on the island of Guadalcanal, consists of a low-K calc-alkaline sequence of 26 different intrusive phases. The major intrusions are characterized by $K/Rb > 400$, $Rb/Sr < 0.06$, $\delta^{18}O$ of 5.7 to 7.2 and uniform $^{87}Sr/^{86}Sr$ of 0.70372. This article presents the first data describing oxygen and strontium isotopic behaviour within a plutonic suite that formed by crystal fractionation.

TECTONIC settings of plutonic rocks (constructive versus destructive margins) in the geological past are often difficult to evaluate because of lack of data from recent plutonic complexes of known tectonic affinities. Such complexes are not commonly found because the very young magmatic terrains must be uplifted and eroded rapidly to expose the plutonic equivalents of the more commonly studied volcanic rocks^{1,2}. Older plutonic complexes (such as the Sierra Nevada batholith) do not provide verifiable constraints on tectonic settings and as such are difficult to use for comparisons. We present new data on an oceanic-arc, sub-volcanic plutonic complex that is found on Guadalcanal in the Solomon Islands. Data from this complex can be used as a reference point for the evaluation of the tectonic setting and mantle–crust affinities of older plutonic complexes.

Geological setting

The Solomon Islands form a double *en echelon* straight arc between New Guinea and the New Hebrides island arc (Fig. 1). At present, subduction is from the south-west, with the Australian Plate being consumed beneath the Pacific Plate; although there is evidence that before the late Miocene the situation was reversed, with the Pacific Plate being subducted from the north-east beneath the Australian Plate³. The Solomon Islands form an oceanic island arc, and Guadalcanal has been near the site of major plate boundaries since at least the Cretaceous. Basement rocks include Cretaceous tholeiitic lavas, and some schistose equivalents, a Palaeocene gabbro, Oligocene–Miocene basaltic andesites (for example, Suta Volcanics) and their plutonic equivalents (Poha Diorite), and Miocene greywackes derived from basic volcanites⁴. The thickness of the basic crust in Guadalcanal is probably between 14 and 20 km (ref. 5).

The Koloula Igneous Complex (Fig. 2) is situated close to the south coast of Guadalcanal, some 50 km from the axis of the South Solomon Trench, which is the topographic expression of a steeply-dipping subduction zone, to which the Pliocene to Recent volcanic centres of Guadalcanal are genetically related⁶. The Koloula Igneous Complex (age: 4.5–1.5 Myr) is considered to have a similar origin, and is exposed solely because of rapid uplift (at least 2 km since the early Pleistocene) and immense erosion caused by seismically-triggered landslides and heavy rainfall, which at present is variable between 7.5 and 15 m yr⁻¹. Despite dense jungle, present exposure is excellent owing to a dense network of deeply-incised streams. Previous studies have dealt with the petrography and porphyry copper mineralization⁷, K–Ar geochronology⁸, fluid inclusions⁹, and mineralogy^{10,11} of the Koloula Igneous Complex.

The Koloula Igneous Complex has intruded the Suta Volcanics of late Oligocene–Miocene age, and consists of a low-K

calc-alkaline sequence of 26 different intrusive phases that has been divided into two major magmatic episodes⁷. Cycle 1 intrusions (≥ 4.5 Myr) are, in order of intrusion: Chakachaka Gabbro (including an olivine pyroxenite cumulate), diorite, Vasuata Quartz Diorite, granodiorite and aplite dykes. The latter two silicic units are volumetrically insignificant whereas the gabbro and the quartz diorite are the largest two intrusions, and together comprise about 80% by area of the complex that is exposed at the present time. The gabbro is composed of plagioclase, augite, hornblende, hypersthene, magnetite and commonly 1–2% quartz; olivine is almost unknown. The quartz diorite contains plagioclase, hornblende, quartz, magnetite, trace clinopyroxene, and typically, at least traces of a graphic quartz–alkali feldspar intergrowth.

Cycle 2 intrusions (2.4–1.5 Myr) are divided into the Inamumu Zoned Pluton (IZP) (Fig. 2) and several satellite intrusions. The latter, and six generations of andesitic and doleritic dykes, are not part of the present study. The IZP is composed of six concentrically-disposed quartz diorite and tonalite units, aplite dykes, the Chikora Tonalite Porphyry, and trondhjemitic dykes (listed in order of intrusion in Table 1). Quartz diorites and tonalites of the IZP contain plagioclase, hornblende, quartz, biotite and magnetite, and except for tonalites 2 and 3, are typically devoid of K-feldspar.

Major- and trace-element data

The variations of major- and trace-element contents of whole rocks versus silica describe smooth and slightly curved trends over the interval 45–76% SiO₂ and are considered to be due to crystal fractionation¹² (Fig. 3). These trends are caused initially by fractionation of amphibole and then by biotite, an observation similar to that noted by Taubeneck¹³ for other tonalitic intrusions. P₂O₅ content exhibits a smooth convex-upward shaped trend, typical in magmatic suites formed by crystal fractionation of apatite¹⁴. The gabbroic rocks are generally strongly leucocratic as they are the residual fraction after the separation of the olivine pyroxenite unit. Their analyses display a much wider compositional scatter than those of the more siliceous rock types and this behaviour is probably related to variable amounts of pyroxene and olivine segregations in some gabbro samples.

With the exception of the late-stage aplite dykes present in both magmatic cycles, there is a compositional overlap of rocks from cycles 1 and 2 between 53 and 64% SiO₂ (based on 110 whole-rock analyses¹²). The major rock-types are characterized by low abundances of K (250–12, 750 p.p.m.), Rb (1–46 p.p.m.), and Ba (207–493 p.p.m.); high Sr (360–700 p.p.m.), moderately high K/Rb and K/Ba ratios and low Rb/Sr ratios. K/Rb and K/Ba ratios are lower for cycle 2 than for cycle 1. Some representative analyses are given in Table 1 and shown in Fig. 4.

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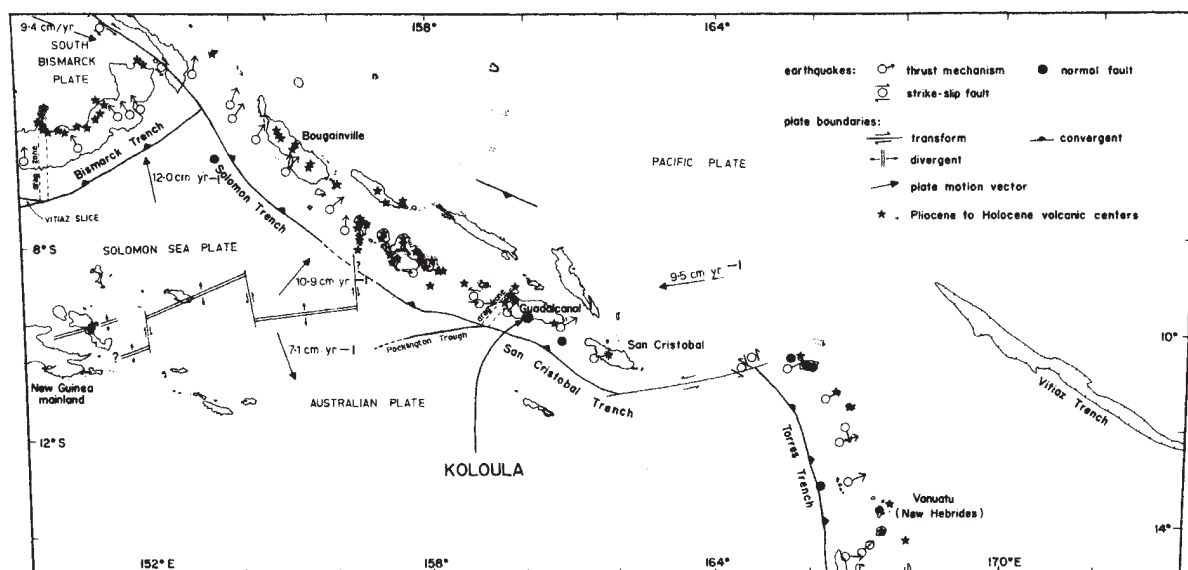


Fig. 1 Location map and tectonic synthesis of the Solomon Islands⁷.

Strontium and oxygen isotope data

Rocks and minerals from plutons belonging to both cycles 1 and 2 have been analysed for oxygen and strontium isotopic compositions (Table 1). The alteration associated with fluid circulation peripheral to known porphyry copper mineralization in the Chikora Tonalite Porphyry⁶ has clearly affected oxygen and hydrogen isotopic compositions in altered rocks. For example, sample A.276, a sericite + calcite + chlorite + pyrite filled fracture has low $\delta^{18}\text{O} = 3.6$ and high $\delta\text{D} = -48$ which are commonly measured values in rocks affected by hydrothermal fluids. Isotopic evidence of hydrothermal alteration can also be seen in some samples (A.628, A.838, A.41) with less obvious petrographic evidence of alteration (see ref. 15). Unlike $\delta^{18}\text{O}$ values, $^{87}\text{Sr}/^{86}\text{Sr}$ ratios from altered samples are the same as values of unaltered rocks or are only slightly higher (0.7037–0.7038). If the alteration fluid were seawater, as has been previously inferred⁷ and as suggested by $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the alteration minerals (laumontite and calcite), then the small shift in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios is a reflection of low water/rock (W/R) ratios and the high strontium contents of the rocks (500–600 p.p.m.) relative to that of seawater (8 p.p.m.)¹⁶. Strontium and oxygen isotope compositions may be used together to determine W/R ratios and temperatures of water–rock equilibration¹⁷. For

closed-system exchange, the strontium W/R (by weight) between seawater and rock, is given by:

$$W/R = \frac{^{87}\text{Sr}/^{86}\text{Sr}^f(\text{rock}) - ^{87}\text{Sr}/^{86}\text{Sr}^i(\text{rock})}{^{87}\text{Sr}/^{86}\text{Sr}^i(\text{H}_2\text{O}) - ^{87}\text{Sr}/^{86}\text{Sr}^i(\text{rock})} \times \frac{C^i(\text{rock})}{C^i(\text{H}_2\text{O})}$$

where i = initial, f = final, $C^i(\text{rock})$ = concentration of strontium in rocks and $C^i(\text{H}_2\text{O})$ = concentration of strontium in seawater. W/R ratios of 4 are calculated for completely altered rocks using data from calcite and laumontite veins where $^{87}\text{Sr}/^{86}\text{Sr}^i = 0.7040$ and the parameters $^{87}\text{Sr}/^{86}\text{Sr}^f(\text{rock}) = 0.7037$, $^{87}\text{Sr}/^{86}\text{Sr}^i(\text{H}_2\text{O}) = 0.709$, $C^i(\text{H}_2\text{O}) = 8$ and $C^i(\text{rock}) = 550$. The strontium contents of altered rocks and minerals (200–400 p.p.m.) are lower than those of unaltered rocks (500–600 p.p.m.) and suggest loss of strontium to the alteration fluid and that the system was not entirely closed. The assumption of closed system behaviour maximizes the calculated W/R ratio. At such low W/R ratios and high concentrations of strontium in the altered rocks, the shifts in $^{87}\text{Sr}/^{86}\text{Sr}$ composition associated with alteration are at the limits of our detection and without the oxygen-isotope data would probably go undetected. As such, use of alteration-related minerals (laumontite, calcite) provides evidence of strontium isotopic exchange that is well within analytical resolution. Using the W/R ratio obtained

Table 1 Geochemical and isotopic data for selected samples from the Koloula igneous complex

	Sample no.	SiO ₂ (%)	Na ₂ O (%)	K ₂ O (%)	Rb (p.p.m.)	Sr (p.p.m.)	Ba (p.p.m.)	Rb/Sr	K/Rb	K/Ba	⁸⁷ Sr/ ⁸⁶ Sr	$\delta^{18}\text{O}$ (‰ SMOW)
Cycle 1												
Olivine pyroxenite	A.A.3	49.54	0.50	0.04	0.7*	164	11	0.004	714	30	0.70374 ± 8	5.6
Chakachaka Gabbro	A.962	46.39	1.80	0.13	2.1*	695*	—	0.003	514	—	0.70374 ± 12	5.4
Vasuta Quartz Diorite	A.628	56.68	4.16	1.09	15	455	207	0.033	603	44	0.70370 ± 8	5.3
Granodiorite	A.838	63.50	4.09	3.20	46	359	493	0.128	577	54	0.70370 ± 8	4.0
Aplite dyke	A.723	75.93	3.77	4.56	64	210	632	0.305	591	60	0.70373 ± 8	7.2
Cycle 2 Inamumu Zoned Pluton												
Charilava Quartz Diorite	A.471	52.83	2.87	0.71	12.8*	59.6*	—	0.021	460	—	0.70378 ± 8	5.7
Charilava Quartz Diorite	A.421	56.30	3.34	0.99	20	602	219	0.033	411	38	0.70371 ± 6	6.0
Tonalite 1	A.356	60.70	3.47	1.16	24	581	274	0.041	401	35	0.70371 ± 8	6.1
Porphyritic tonalite	A.118	62.71	3.80	1.39	31	626	327	0.050	540	35	0.70376 ± 12	6.2
Tonalite 2	A.84	64.02	3.63	1.25	27	652	351	0.041	384	30	0.70368 ± 8	6.7
Tonalite 3	A.82	65.24	3.77	1.53	37	635	409	0.058	343	31	0.70374 ± 8	6.6
Aplite dyke	A.6	76.67	3.09	4.93	58	203	765	0.286	1,024	53	0.70372 ± 20	6.9
Chikora Tonalite Porphyry	A.41	67.77	4.42	0.87	—	—	—	—	—	—	0.70363 ± 10	6.1
Trondhjemite dyke	CH.8-622.5	75.83	4.58	1.10	—	—	—	—	—	—	—	7.2
Altered rocks and minerals												
Pervasive sericite–calcite–chlorite–pyrite zone in Charilava Quartz Diorite	A.276	51.14	0.81	3.79	107	191	427	0.560	294	74	0.70376 ± 8	3.6
Laumontite vein	A.479	—	—	—	—	380*	—	—	—	—	0.70404 ± 8	—
Calcite vein	IHP.493	—	—	—	—	—	—	—	—	—	0.70401 ± 12	—

* Concentration determined by isotope dilution. All other abundances determined by X-ray fluorescence analysis. Strontium isotopic ratios have been normalized to E & A standard value of 0.7080; errors are reported as standard error of the mean at 2σ .

Table 2 Comparative geochemical and isotopic data for rocks from oceanic and continental terrains

		SiO ₂ (%)	K (p.p.m.)	Rb (p.p.m.)	Sr (p.p.m.)	Rb/Sr	K/Rb	⁸⁷ Sr/ ⁸⁶ Sr	Refs
Oceanic									
Koloula	Cycle 1								
	Gabbro	46.4	1,079	2.1	695	0.003	514	0.70374	
	Quartz diorite	56.7	9,049	15	455	0.033	603	0.70370	
	Cycle 2								
	Tonalite	60.7	9,628	24	581	0.041	401	0.70358	
	Gabbro	43.1	585		280	0.0035	585	0.70345	21
Tanzawa	Tonalite	62.1	4,553	8	251	0.032	569	0.70367	
Viti Levu	Gabbro	48.7	1,410	2	190	0.010	705	0.7037	22
	Tonalite	64.4	5,560	8	349	0.023	695		
Captains Bay	Gabbro	48.4	996	3	640	0.005	333	0.70377	24
	Quartz diorite	59.3	12,450	34	426	0.08	368	0.70317	
Continental									
Chile (Tertiary)	Tonalite	62.5	13,860	45	465	0.10	308		26
USA Cloudy Pass	Quartz diorite	61.4	19,670	65	288	0.23	303	0.7047	29

above and the equation for closed-system oxygen isotope exchange of seawater and rock (by weight)¹⁵:

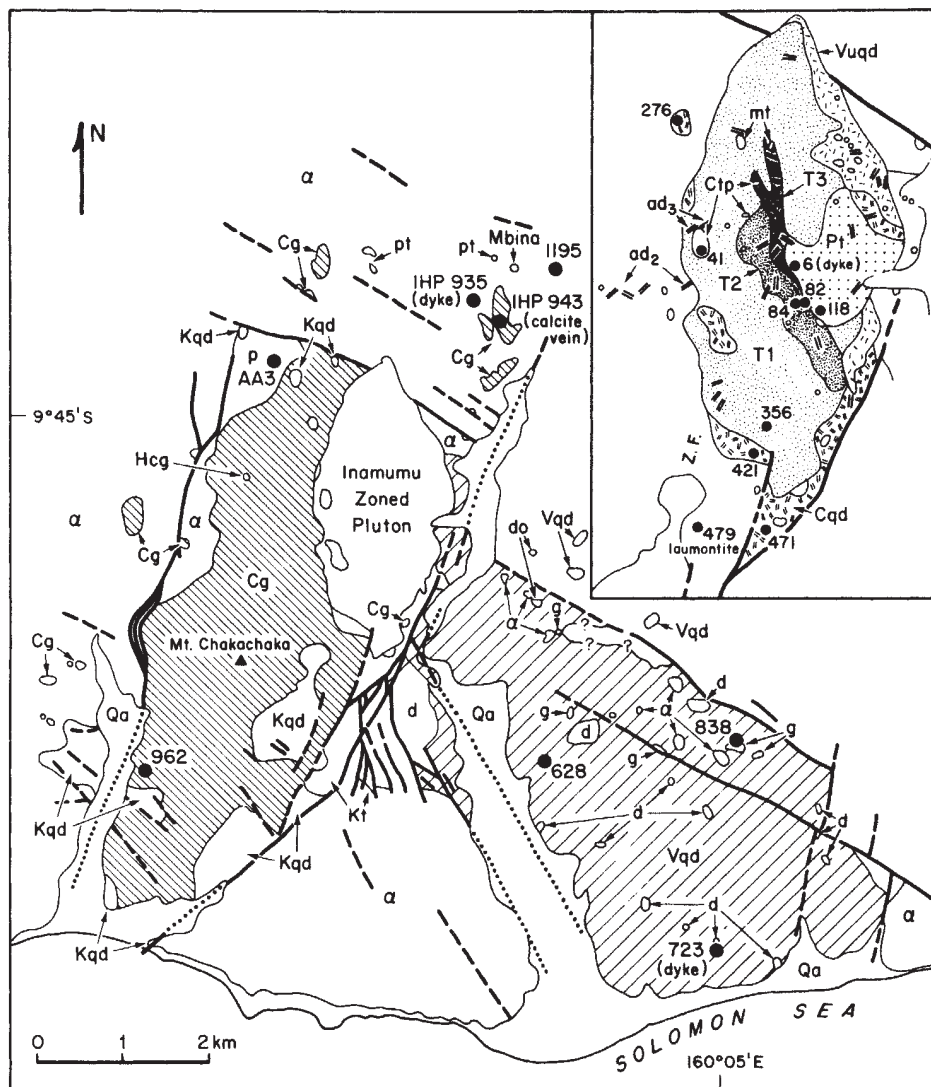
$$W/R = \frac{\delta^i(\text{rock}) - \delta^i(\text{rock})}{\delta^i(\text{H}_2\text{O}) - (\delta^i(\text{rock}) - \Delta)} \times \frac{C^i(\text{rock})}{C^i(\text{H}_2\text{O})}$$

we can obtain values for Δ . For the alteration of the Charilava Quartz Diorite with seawater we obtain a value of $\Delta = 3.4$. Using the feldspar-H₂O geothermometer¹⁸ ($\Delta = 2.68 (10^6/T^2) - 3.53$) we calculate a temperature of 350 °C for water-rock equilibration. This agrees well with the fluid inclusion filling temperature of 350 °C obtained on rocks from the same B4 alteration zone⁹.

Using petrographic criteria and oxygen isotope compositions to eliminate altered samples, the unaltered rocks from cycle 1 have $\delta^{18}\text{O}$ values that range from 5.4 for a gabbro to 7.2 for an aplite dyke whereas $\delta^{18}\text{O}$ values from the IZP (cycle 2) range from 5.7 (quartz diorite) to 7.2 (trondjemite dyke). The observed variations in $\delta^{18}\text{O}$ values (5.4–7.2) and SiO₂ contents (46–77%) fall along a well defined line with a slope of 0.06‰/% SiO₂ (Fig. 5). For these samples, $^{87}\text{Sr}/^{86}\text{Sr}$ ratios range from 0.70368 to 0.70378 ($\bar{x} = 0.70372 \pm 8$ at 2σ) for the entire range of SiO₂ and are identical within experimental errors.

The lack of variation in strontium isotopic ratios and the correlation of $\delta^{18}\text{O}$ with degree of differentiation (given by %

Fig. 2 Geological map of the Koloula Igneous Complex showing sample locations. Faults are shown as both heavy dotted and solid lines. Cycle 1 rock types: Cg, Chakachaka Gabbro; p, olivine pyroxenite; d, diorite; Vqd, Vasuata Quartz Diorite; do, dolerite; g, granodiorite. Cycle 2 satellite plutons: Kqd, Kolochoro Quartz Diorite; Kt, Kolokemau Tonalite; Hcg, High Chikora Granodiorite. α , Suta Volcanics (Oligocene to early Miocene); Qa, Holocene alluvium; Q, Inamumu Zoned Pluton (inset): Vuqd, Vurakara Quartz Diorite; Cqd, Charilava Quartz Diorite; T1, tonalite 1; Pt, porphyritic tonalite; T2, tonalite 2; T3, tonalite 3; mt, melanocratic tonalite; Ctp, Chikora Tonalite Porphyry. Aplite and hornblende andesite dykes are not shown. ZF, zone of foliation.



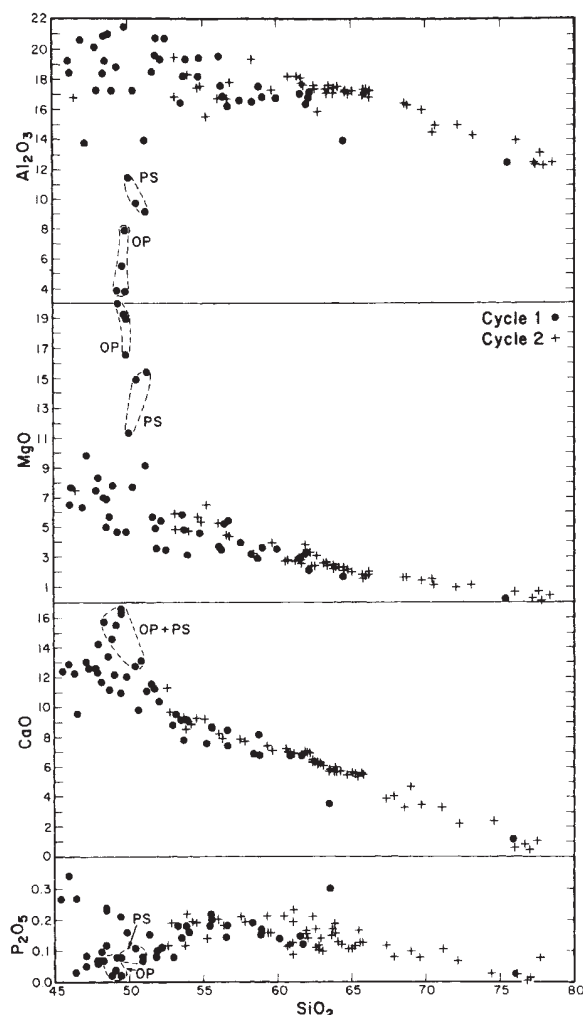


Fig. 3 Harker variation diagrams for selected major elements for samples from the Koloula Igneous Complex. OP, olivine pyroxenite; PS, pyroxenite segregation. Five samples of unusually mafic-rich gabbro provide a trend of high P_2O_5 contents between $SiO_2 = 45\%$ and 50% .

SiO_2) is indicative of temperature-dependent fractionation of oxygen isotopes. The magnitude of oxygen isotope fractionation between cumulus and liquid phases is dependent on the temperature of crystallization and the mineralogy of the cumulate phases in equilibrium with the liquid. As such, the slope of the correlation line on the SiO_2 versus $\delta^{18}O$ diagram for igneous rocks that have formed by simple crystal fractionation, will not be the same for all plutonic and volcanic complexes. However, for all igneous suites that have originated in the mantle, the correlation line should pass through a common field. In a suite of tholeiitic lavas from the Hachijo-jima volcano in the Izu-Mariana arc, Matsuhisa¹⁹ found a $\delta^{18}O$ variation from 5.7 to 6.7‰ concomitant with a variation in SiO_2 of 47–73% (0.04‰/% SiO_2).

Comparisons with other epizonal plutons

Although well documented studies of subvolcanic magma chambers are rare, we have selected five examples that are probably related to arc magmatism (Table 2). Two of the areas are considered oceanic, two are continental and one, transitional from oceanic to continental. The oceanic plutonic complexes from Tanzawa, Japan and Viti Levu, Fiji are similar to the Koloula complex, in rock type (gabbro-tonalite) and geochemical data (Fig. 4). The Tanzawa complex is composed primarily of gabbro-tonalite plutons of Miocene age and has been interpreted as a low-K, island-arc plutonic complex^{20,21}. The strontium isotope data from Tanzawa appear to be slightly anomalous in that the tonalites have an average $^{87}Sr/^{86}Sr$ ratio

of 0.7036 whereas the gabbros give values of 0.70345. Although data are available from Viti Levu, it is obvious that significant alteration (10–20 modal % of alteration minerals) has caused considerable scatter²². Other geochemical characteristics (K, Rb, Sr contents) of the igneous complexes from both Tanzawa and Viti Levu are consistent with their having been derived from oceanic mantle with no crustal component. Measured K/Rb (>400) and Rb/Sr (<0.06) ratios are similar to volcanic rocks from other oceanic island arcs (for example, Tonga²³). The Captains Bay pluton, Unalaska Island, Alaska²⁴ is zoned inward from gabbro to monzoniorite. Even though the $^{87}Sr/^{86}Sr$ ratios are similar to Koloula (0.70377), the low K/Rb (333–368) and the high Rb/Sr (0.08) for the quartz diorite are different. Based on conclusions of Perfit *et al.*²⁴ we have included the data under our oceanic plutonic complexes, but it is likely that Unalaska Island may straddle the continental–oceanic environments²⁵.

The continental-margin plutonic complexes related to subduction from both Chile²⁶ and western USA (Cloudy Pass²⁷ and Tatoosh^{28,29}) are mostly tonalitic in composition and show consistently high K (>13,000 p.p.m.) and Rb (>42 p.p.m.) contents, high Rb/Sr (>0.08), and low K/Rb (<333). Lines with a Rb/Sr value of 0.06 and K/Rb ratio of 400 can be drawn in Fig. 4 that effectively separate oceanic and continental terrains. The limited strontium isotopic data (>0.7040) suggests a crustal component in the evolution of these plutons.

Except for Captains Bay, there is a notable lack of stable-isotope data for the examples cited so that we have little indication of the degree of alteration and its effects on other geochemical parameters. The large variation of $\delta^{18}O$ values from Captains Bay²⁴, of –4.1 to +5.7 is reflective of variations in $^{87}Sr/^{86}Sr$ ratios of 0.70328–0.70377 which can be caused by hydrothermal alteration.

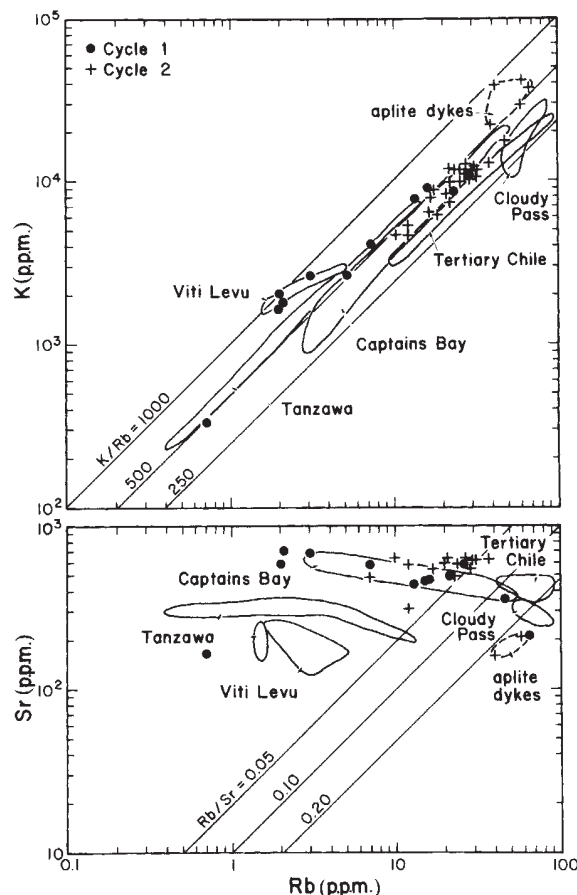


Fig. 4 K–Sr–Rb diagram for the Koloula Igneous Complex. Also shown are fields of gabbro to tonalite rocks from Tanzawa²¹, Viti Levu²², Captains Bay²³, Tertiary tonalites from Chile²⁶ and Cloudy Pass²⁹.

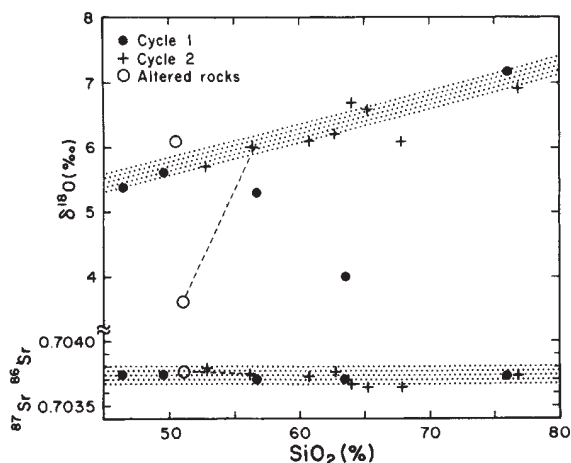


Fig. 5 Variation in whole-rock $\delta^{18}\text{O}$ values and initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios with % SiO_2 for samples from the Koloula Igneous Complex. Petrographic observations were used to characterize alteration. Tielines join altered and unaltered samples from the same intrusive phase.

Interpretation

The Koloula Igneous Complex is composed of 26 intrusive phases that range from gabbro to aplite. The youthfulness of the complex, its excellent exposure and our knowledge of its tectonic origins make the Koloula study unique in the geological literature. The geochemical variations seen within the two cycles of igneous intrusion are considered to be due to crystal fractionation. Within each cycle K/Rb and K/Ba ratios are similar and suggest that the members of each cycle are comagmatic. The difference in K/Rb and K/Ba ratios between cycles, with no

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1. Jakes, P. & White, A. J. R. *Bull. geol. Soc. Am.* **83**, 29–40 (1972).
2. Ewart, A., Brothers, R. N. & Meehan, A. J. *Volcanol. Geotherm. Res.* **2**, 205–250 (1977).
3. Kroenke, L. W. *Hawaii Inst. Geophys., Univ. Hawaii Rep. HIG-72-5* (1972).
4. Hackman, B. D. thesis, Univ. Western Australia (1971).
5. Furumoto, A. S. et al. *Pacif. Sci.* **24**, 315–332 (1970).
6. Chivas, A. R. *Bull. Aust. Soc. Explor. Geophys.* **6**, 64–65 (1975).
7. Chivas, A. R. *Econ. Geol.* **73**, 645–677 (1978).
8. Chivas, A. R. & McDougall, I. *Econ. Geol.* **73**, 678–689 (1978).
9. Chivas, A. R. & Wilkins, R. W. T. *Econ. Geol.* **72**, 153–169 (1977).
10. Chivas, A. R. *Contr. Miner. Petrol.* **78**, 389–403 (1981).
11. Hendry, D. A. F., Chivas, A. R., Reed, S. J. B. & Long, J. V. P. *Contr. Miner. Petrol.* **78**, 404–412 (1981).
12. Chivas, A. R. thesis, Univ. Sydney (1977).
13. Taubeneck, W. H. J. *geol. Soc. Am. Spec. Pap.* **91**, 1–56 (1967).
14. White, A. J. R. & Chappell, B. W. *Tectonophysics* **43**, 7–22 (1977).
15. Taylor, H. P. Jr *Econ. Geol.* **69**, 843–883 (1974).
16. Faure, G. & Powell, J. L. *Strontium Isotope Geology* (Springer New York, 1972).

difference in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and $\delta^{18}\text{O}$ values suggests a similar source for the magmas but different degrees of partial melting.

The large range in composition and relatively low and constant $^{87}\text{Sr}/^{86}\text{Sr}$ ratios observed in these rocks also makes it possible to model variations in oxygen isotope composition as related to crystal fractionation. Although such variations have been noted in volcanic rocks from Hachijo-Jima in the Izu-Mariana group²¹, our data are the first to demonstrate these isotopic systematics in plutonic rocks resulting purely from crystal fractionation of a mantle-derived magma in a closed system. From our data which display relatively low $\delta^{18}\text{O}$ at high SiO_2 contents it becomes increasingly evident that examples of the more potassic suite, gabbro $\rightarrow$ granodiorite $\rightarrow$ granite are not a result of closed-system differentiation in either small (for example Ploumanac'h³⁰) or large plutonic bodies (for example, Peninsular Ranges³¹). The evolution of these granites involves magma contamination by continental crust or partial melting of mixed mantle/continental source regions. Volcanic rocks from the Banda Arc also exhibit a correlation of $^{86}\text{Sr}/^{86}\text{Sr}$ with $\delta^{18}\text{O}$ that has been interpreted as being the result of contamination by crustal material or by subducted sediments³².

From the comparisons with the limited data available from other young island-arc plutonic complexes, it appears that in unaltered complexes distinctions that are based on trace-element chemistries and isotopic ratios may be made between oceanic and continental environments.

Before this can be applied rigorously to plutonic complexes of uncertain tectonic environment, we need many more detailed studies of young complexes in known tectonic terrains. When this data base is established it may be then applied to older orogens where plutonic rocks are commonly the only remnants of volcanic activity.

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17. McCulloch, M. T., Gregory, R. T., Wasserburg, G. J. & Taylor, H. P. Jr *Earth planet. Sci. Lett.* **46**, 201–211 (1980).
18. O'Neil, J. R. & Taylor, H. P. Jr *Am. Miner.* **52**, 1414–1437 (1967).
19. Matsuhisa, Y. J. *Volcanol. Geotherm. Res.* **5**, 271–296 (1979).
20. Takita, R. J. *geol. Soc. Jap.* **80**, 505–523 (1974).
21. Ishizaka, K. & Yangi, T. *Earth planet. Sci. Lett.* **33**, 345–352 (1977).
22. Gill, J. B. *Contr. Miner. Petrol.* **27**, 179–203 (1970).
23. Ewart, A., Bryan, W. & Gill, J. J. *Petrol.* **14**, 429–465 (1973).
24. Perfit, M. R., Brueckner, H., Lawrence, J. R. & Kay, R. W. *Contr. Miner. Petrol.* **73**, 69–87 (1980).
25. Marsh, B. D. in *Andesites: Orogenic andesites and related rocks* (Thorpe, R. S.) (Wiley, New York, 1982).
26. Lopez-Escobar, L., Frey, F. A. & Oyarzun, J. *Contr. Miner. Petrol.* **70**, 439–450 (1979).
27. Cater, F. W. *U.S. geol. Surv. Spec. Pap.* **116**, 1–54 (1969).
28. Fiske, R. S., Hopson, C. A. & Waters, A. C. *U.S. geol. Surv. Prof. Pap.* **444**, 1–93 (1963).
29. Meijer, A. thesis, Univ. California, Santa Barbara (1971).
30. Albarède, F., Dupuis, C. & Taylor, H. P. Jr *J. geol. Soc. Lond.* **137**, 641–647 (1980).
31. Silver, L. T., Taylor, H. P. Jr & Chappell, B. W. *Geol. Soc. Am. A. Meet. Field Trip Guide* 83–110 (San Diego State University, California, 1979).
32. Magaritz, M., Whitford, D. J. & James, D. E. *Earth planet. Sci. Lett.* **40**, 220–230 (1978).

Mechanism of activation of a human oncogene

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The oncogene of the human EJ bladder carcinoma cell lines arose via alteration of a cellular proto-oncogene. Experiments are presented that localize the genetic lesion that led to activation of the oncogene. The lesion has no effect on levels of expression of the oncogene. Instead, it affects the structure of the oncogene-encoded protein.

A LARGE body of work on chemical carcinogenesis has demonstrated that the carcinogenic potency of a compound is correlated with its mutagenic powers¹⁻⁴; this suggests that DNA is the ultimate target of carcinogenic activation. Accordingly, we and others initiated a series of experiments designed to identify and study DNA segments in tumour cells whose alteration is critically important for oncogenic conversion⁵⁻⁹.

An initial approach to this problem involved the transfer of tumour cell DNA into non-transformed NIH 3T3 mouse fibroblasts. Such experiments indicated that the phenotype of cellular transformation could be passed from cell to cell in this manner. Thus, the tumour DNA was able to induce foci of transformed cells in a recipient NIH 3T3 monolayer culture while DNA of normal, untransformed donor cells failed to do so⁵⁻⁹. These results demonstrated the existence of oncogenic factors which were present in tumour cell DNA and apparently absent from the DNA of normal cells.

That specific donor DNA sequences were involved in these phenomena was first indicated by studies examining the sensitivity or resistance of the biological activity of the DNA to treatment by various site-specific endonucleases^{10,11}, and was later directly demonstrated by molecular isolation of discrete transforming genes from human bladder carcinoma cell lines¹²⁻¹⁴. Use of clones of the bladder oncogene as sequence probes showed that the oncogene derived from a sequence of similar structure present in the normal human genome¹²⁻¹⁴. It appeared, therefore, that the bladder oncogene had arisen by the mutation of a normal cellular gene during the process of

carcinogenesis. The present report addresses the nature of the alterations that activated the oncogene of the EJ human bladder carcinoma cell line.

Comparison of this EJ bladder oncogene with the related cellular sequence (the proto-oncogene) was aided greatly by the subsequent discovery that this oncogene was homologous to the transforming gene of the rat-derived Harvey murine sarcoma virus¹⁵⁻¹⁷. This rat sarcoma virus gene, termed *v-Ha-ras*, had been acquired from the rat genome during the process of formation of the chimaeric viral genome^{18,19}. Both the rat and human cellular homologues of *v-Ha-ras* have been isolated in the course of studies of this gene^{20,21}. The human cellular homologue of the *v-Ha-ras* was found to correspond precisely to the normal antecedent of the EJ bladder oncogene¹⁶.

From examination of the data in these cited reports, it was possible to make preliminary comparisons between the EJ oncogene¹³ and its normal cellular counterpart (termed *c-Ha-ras*)²². For example, a molecular clone of the normal cellular gene did not induce foci when applied to NIH 3T3 monolayers, while a clone of the bladder oncogene exhibited a biological activity of $\sim 5 \times 10^4$ focus-forming units per μg of transfected DNA^{13,21}. This stark difference in function did not correlate with any apparent structural differences between the two clones. Rough restriction endonuclease site mapping of the EJ oncogene clone and the uncloned related human proto-oncogene indicated that the two were basically indistinguishable over the 6.6 kilobase (kb) of sequence which contained the transforming activity of the EJ oncogene¹³. Finer mapping was later made possible by the direct comparison of molecular clones of the two genes. We again found no differences using a series of different endonucleases, with the exception of a single difference 3' (downstream) of the coding regions of the genes (data not shown). This latter difference was interpreted to represent a functionally silent polymorphism of the gene present in the human gene pool. Such polymorphisms of this gene have been documented independently by others¹².

These results presented an apparent paradox, since a drastic functional difference was exhibited by two structurally similar genes. We assumed that a minor alteration was responsible for the functional difference, and that this alteration could affect function in one of two ways: the alteration could involve a change in sequences regulating the expression of the gene; alternatively, the transformed phenotype could be due to changes in the protein-encoding portion of the gene. The first hypothesis would argue for up-regulation of transcription or translation of the gene, yielding high levels of an otherwise normal protein product, while the second model would suggest synthesis of an altered protein. Both types of alteration could also act in concert to create the observed difference in function.

Analysis of oncogene activity in normal and transformed bladder cells

If the significant difference between the oncogene and proto-oncogene were one of regulation, one would expect to see differences in levels of RNAs and proteins specified by these genes. To address this question, we first examined the EJ bladder carcinoma cells and their counterpart normal cells. Normal bladder epithelial cells were cultured from a specimen of normal human bladder as described²³, and were found to synthesize keratins, require a fibroblast feeder layer support, and to possess a limited lifespan in culture, as would be expected for normal epithelial cells (T. O'Connell and J. G. Rheinwald, personal communication). The cultures were free of human stromal fibroblasts, and were purified free of feeder cells before nucleic acid extraction. These cultured cells therefore provided a source of genetic material and RNA transcripts that were the normal counterparts of the bladder carcinoma genes and transcripts.

Total cellular RNA was prepared from both normal and transformed bladder cells. Transcripts were analysed by running the RNA on a formaldehyde gel, transferring it to a nitrocel-

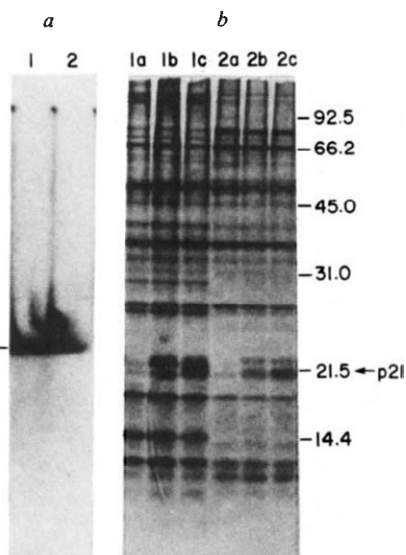


Fig. 1 Comparison of expression of the *c-Ha-ras* gene in normal human bladder epithelial cells with that in the EJ transformed bladder cell line. The normal bladder epithelial cells, strain HB1-5, were secondary and tertiary passage cultures derived from explants of a 5-month fetal human bladder. The cells were cultured as described²³. **a**, The relative levels of *c-Ha-ras* specific RNA in the two cell types: lane 1, RNA from EJ cells; lane 2, RNA from HB1-5 cells. Total polyadenylated RNA was prepared by the technique of Varmus *et al.*⁴⁰; 4 μg of RNA was then fractionated by electrophoresis through formaldehyde-containing 2% agarose gels and transferred to nitrocellulose (B. Seed & D. Goldberg, manuscript in preparation). A *ras*-specific probe was prepared by cutting pEJ with *Bam*HI, fractionating the resultant fragments through a 1% agarose gel and extracting the 6.6-kb insert with NaI and glass beads. The nick-translated fragment (6.6×10^7 c.p.m. μg^{-1}) was annealed to the immobilized RNA^{41,42}. Bands hybridizing to the probe were visualized by autoradiography. Molecular weights were determined by comparison with markers obtained from *in vitro* run-off transcription of the adenovirus late promoter⁴³. **b**, A comparison of p21 proteins immunoprecipitated from cell lysates of EJ cell clones (lanes 1, a-c) and HB1-5 cells (lanes 2, a-c). Cultures were labelled with ³⁵S-methionine for 12 h. Lysates were then prepared and immunoprecipitated with non-immune serum (lanes 1a and 2a), a monoclonal antiserum (Y13-238) which precipitates the p21 encoded by Ha-MuSV but not the p21 encoded by Ki-MuSV (lanes 1b and 2b) or a monoclonal antiserum (Y13-259) which detects both the Ha-MuSV and the Ki-MuSV p21s (lanes 1c and 2c)^{25,32}. 20×10^6 c.p.m. of lysate per sample were resolved by electrophoresis through a 12.5% SDS-polyacrylamide gel.

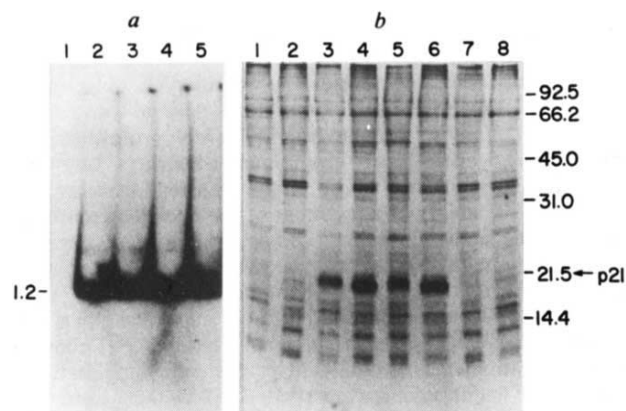


Fig. 2 Analysis of cells transfected with molecular clones of the EJ transforming gene (pEJ) or its normal cellular homologue (pEC). Transfections were carried out as previously described^{44,45} using 75 µg NIH 3T3 carrier DNA, 500 ng pEJ or pEC DNA, and 50 ng pEcogpt DNA per 2×10^6 cells. Colonies resistant to mycophenolic acid were selected²⁶. **a**, Analysis of total polyadenylated RNA from the four transfected cell lines. RNAs were isolated and analysed as described in Fig. 1. Lane 1, RNA from NIH 3T3 cells; lane 2, EJ/Gpt-2 cells; lane 3, EJ/Gpt-3 cells; lane 4, EC/Gpt-1 cells; lane 5, EC/Gpt-5 cells. **b**, A comparison of p21 proteins immunoprecipitated from cell lysates of the four lines transfected with pEJ or pEC. Cell lysates were prepared, immunoprecipitated and analysed as described in Fig. 1 legend. Immunoprecipitations were performed with non-immune serum (lanes 1, 2, 7, 8) or the monoclonal antiserum (Y13-238) which precipitates the Ha-MuSV p21 (lanes 3-6). Cell lysates were prepared from EJ/Gpt-2 (lanes 1, 3); EC/Gpt-1 (lanes 2, 4); EJ/Gpt-3 (lanes 5, 7) and EC/Gpt-5 (lanes 6, 8).

lulose filter, and probing the filter with nick-translated EJ oncogene clone. Figure 1a shows that similar levels of RNA were detected in the two cultures. The transcripts were of a size (1.2 kb) previously associated with the *c-ras* genes of other cell types²⁴.

The only known products of the *ras* genes are proteins of molecular weight ~21,000, referred to as p21. Monoclonal antisera against the v-Ha-*ras* p21 protein²⁵ were used to precipitate metabolically labelled protein lysates from both EJ and normal bladder cells. Control experiments ensured that the amounts of antibody used in this and subsequent experiments were in excess of that required to immunoprecipitate the antigen present. The data shown in Fig. 1b indicate that at least two bands of radiolabelled protein were specifically precipitated by the anti-p21 sera from normal bladder cells. Detailed examination of the protein pattern of the bladder carcinoma seen in this and other gels reveals a more complex array of bands. On close inspection we believe that four bands in total can be discerned. Data to be presented below clarify the origin of the novel p21 bands seen in the EJ tumour cells. After comparing the intensities of the p21 bands with those of non-specifically precipitated background bands, it became apparent that the total amount of p21 proteins of the normal and the tumour cells differed less than threefold.

It thus seems that increased levels of transcription are not responsible for the novel activity exhibited by the EJ oncogene. This conclusion rests in part on the fact that in the hybridization conditions used here, the oncogene probe reacts exclusively with transcripts of the human *c-Ha-ras-1* gene. Interpretation of the protein data is less clear. It is apparent that both cells have comparable levels of proteins that are reactive with the Harvey-specific serum, and that these proteins can be collectively termed 'p21'. The nature of these proteins can only be addressed by other studies, presented in part below.

Analysis of gene activity in cells transfected with cloned DNAs

It remained possible that the bladder epithelial cells were not, as we believed, representatives of the normal precursors of the bladder carcinoma cells. Such a possibility might cloud interpre-

tation, as a *ras* gene could be expressed at a high level in one cell type without inducing transformation, and only achieve this phenotype when inappropriately expressed in a second cell type. We therefore wished to measure the levels of transcription and translation of the two genes in the same cellular background.

We therefore introduced molecular clones of both genes into NIH 3T3 cells. Colonies acquiring the EJ oncogene could be readily identified by their transformed morphology. However, cells acquiring clones of the normal allele were not identifiable by any obvious change in behaviour. Because of this, we co-transfected a clone of the dominant selectable *Ecogpt* gene²⁶ into NIH 3T3 cells together with a 10-fold excess of either the cloned EJ oncogene (pEJ) or the cloned proto-oncogene (pEC). In each case, colonies were selected for resistance to mycophenolic acid imparted by acquired *Ecogpt* genes²⁶. This strategy was used since the introduction of a non-selected DNA segment can be ensured by co-transfection with a selectable gene²⁷. In the present case, 75% of the mycophenolic acid-resistant colonies deriving from co-transfection of *Ecogpt* and pEJ were seen to be morphologically transformed; as expected, none of the colonies emerging after co-transfection with pEC was transformed.

The cellular DNA of both classes of colonies was analysed for the presence of pEC or pEJ sequences. DNA was cleaved with the restriction enzyme *Bam*HI, which would be expected to liberate a 6.6-kb fragment from each intact copy of the cloned oncogene or proto-oncogene. The normal mouse homologue of the *ras* gene hybridizes only weakly to the pEJ probe¹⁶, and so its presence does not obscure the analysis. To ensure that transfected pBR322 sequences would not interfere with interpretation of the data, *ras*-specific sequences were prepared from pEJ and used as probe; 75% of non-transformed colonies transfected with the proto-oncogene and all of the transformed, oncogene-transfected colonies showed the presence of pEJ-homologous sequences migrating at 6.6 kb (data not shown).

We selected for further analysis two cell lines containing intact copies of the oncogene and two lines containing an approximately equal number of intact copies of the proto-oncogene. Photographs of these cell lines are shown in Fig. 3; 4×10^6 cells of each of these lines were injected into 6-week-old NSF mice. Each of the pEJ transfected lines elicited tumours in 5/5 mice, while none of the mice injected in parallel with pEC transfectants developed tumours.

Total cellular RNA was prepared from all four transfected cell lines. The RNA preparations were then run on a formaldehyde gel, transferred to nitrocellulose filters, and probed with the *ras*-specific DNA. As shown in Fig. 2a, the level of *ras*-specific RNA in cells containing the oncogene was comparable with those carrying the transfected proto-oncogene. We also examined the levels of p21 in these cell lines. Figure 2b shows that monoclonal antiserum against p21 precipitates similar amounts of p21 protein in pEC and pEJ transfected cells.

These data do not address the question of whether the two cloned genes are transcribed at the same rates in these cells, as it remains formally possible that few of the acquired copies of the pEJ are active in these particular pEJ-transfected cells, while all copies of the transfected pEC gene in the other cells might be active. In such a case, the comparable levels of protein and RNA observed would not accurately reflect the intrinsic transcriptional activities of the two genes.

However, one point emerges with clarity from these experiments: a certain level of EJ-specified p21 induces transformation, while a comparable if not higher level of the proto-oncogene-specified p21 has no effect on cellular phenotype. Since the p21 proteins are the only apparent gene products encoded by these genes, we conclude that the difference in function between the EJ oncogene and the proto-oncogene must derive from structural alterations in the p21 protein. Conversely, regulatory alterations do not seem critical to the transforming activity of the oncogene.

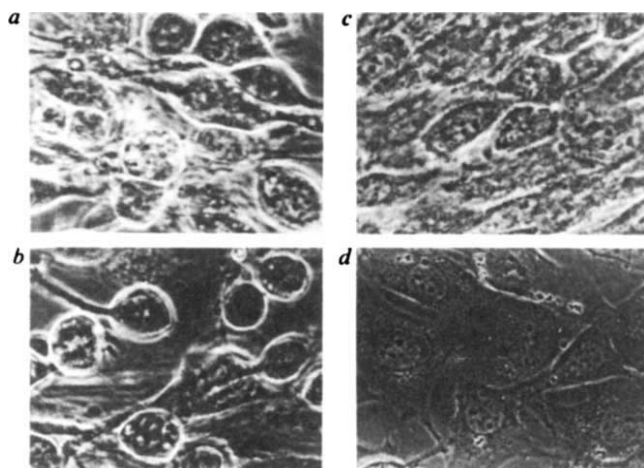


Fig. 3 Photographs of cell lines transfected with pEJ or pEC through a phase contrast microscope ($\times 300$). Cell lines were derived as described in Fig. 2 legend. Cell lines transfected with pEJ are shown at confluence (EJ/Gpt-2, a) and subconfluence (EJ/Gpt-3, b). Cell lines transfected with pEC are also shown at confluence (EC/Gpt-5, c) and subconfluence (EC/Gpt-1, d).

Detection of differences in the size of the oncogene product

In view of the above conclusion, new importance was attached to the previously detected slight variations in migration rates of the p21 proteins from different cells (Figs 1b and 2b). We therefore re-analysed the p21 proteins after shorter periods of metabolic labelling, which we expected would highlight these differences more clearly. Data shown in Fig. 4a indicate that the pEJ and pEC transfectants each exhibit two bands of p21. Kinetic labelling experiments indicate that in each case the more slowly migrating band behaves as a kinetic precursor to the more rapidly migrating band (manuscript in preparation). Comparable data on the p21 protein of v-Ha-ras previously indicated that the higher band underwent post-translational cleavage to yield its lower, more rapidly migrating partner²⁸.

Further examination of Fig. 4a reveals that the higher molecular weight p21 protein of the pEJ transfectant migrates slower than the higher molecular weight protein of the pEC transfectant and also that the lower molecular weight p21 of the pEJ transfectant migrates slower than the lower molecular weight p21 of the pEC transfectant. The relative migration rates of these proteins, and the relationships we impute to them from kinetic data and previously published experiments²⁸ are indicated schematically in Fig. 4a.

As none of the p21 proteins seems to be phosphorylated to any extent (data not shown), the differences in migration between the pEC and pEJ proteins are most readily attributed to alterations in the number of amino acids or to changes in conformation. The data and the schematization of Fig. 4a may provide an explanation for the complexity of p21 proteins seen in normal and transformed bladder cells (Fig. 1b): the normal cells exhibit two bands, reflective of the expression of a proto-oncogene; the carcinoma cells appear to exhibit four bands, two being specified by the oncogene of these cells, and two by the normal, proto-oncogene of the other, homologous chromosome.

Genetic mapping of the functionally altered region of the oncogene

To determine whether the observed physical difference between p21 proteins reflected a functionally important change involved in the process of transformation, we set out to supplement these results with an independent series of experiments designed to localize genetically the regions of the oncogene that specify the altered migration rate of the protein and the change in gene function. These experiments depended on *in vitro* homologous

recombination between clones of the two genes. The experimental design was to excise a restriction fragment out of the oncogene and use it to replace the homologous piece of the proto-oncogene. At the same time, the reciprocal construction was carried out by splicing the fragment of the proto-oncogene into the oncogene. These recombinant constructs were then tested for transforming ability in the transfection assay. We looked for the ability of a fragment of the oncogene to impart transforming activity when placed in the midst of a proto-oncogene clone, and conversely, in the reciprocal recombination, for the loss of activity when the corresponding proto-oncogene fragment was inserted into the oncogene clone. The constructions are shown schematically in Fig. 5. Following *in vitro* ligation, the recombinant clones were amplified and mapped to confirm their construction. Only then were they transfected to NIH 3T3 monolayers.

It was vital that we should be able to verify that the transforming clones were indeed chimaeras of mixed pEJ and pEC origin, rather than contaminants of one origin or the other. This was

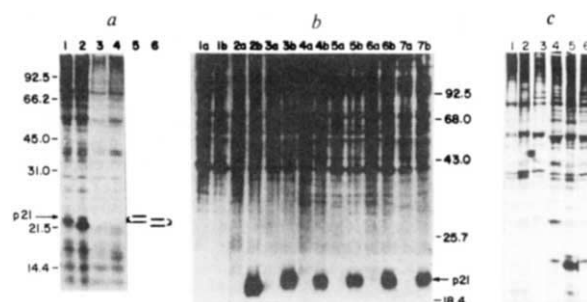
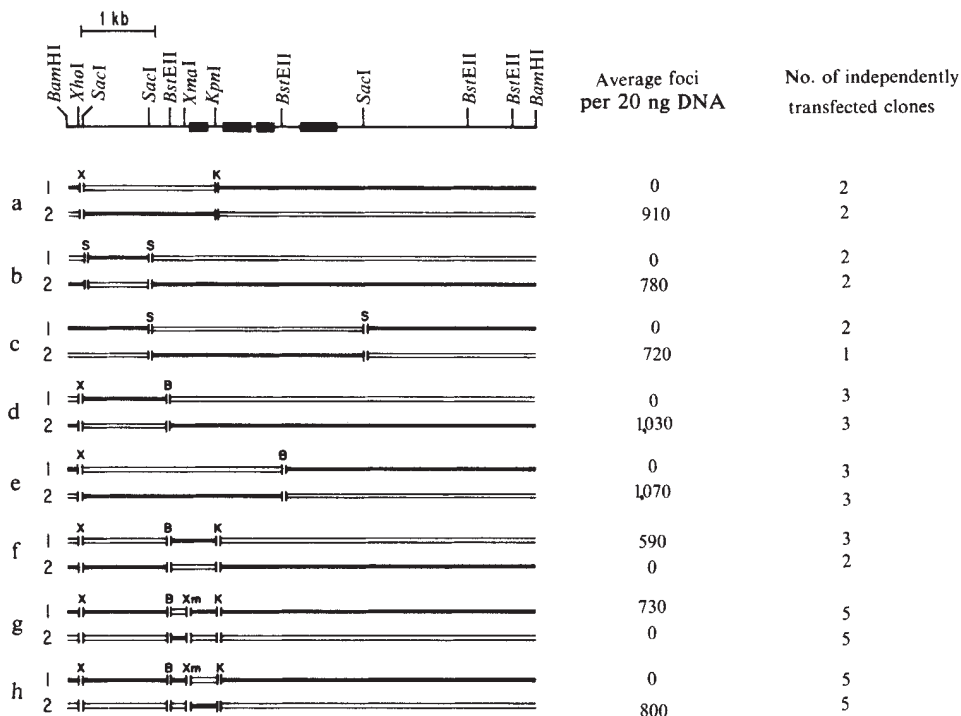


Fig. 4 Comparison of the mobility of p21 proteins encoded by the EJ bladder carcinoma transforming gene and its normal homologue. **a**, Immunoprecipitations from cells transfected with pEJ or pEC. Cells were metabolically labelled with ^{35}S -methionine for 3 h; cell lysates were prepared, immunoprecipitated and analysed as described in Fig. 1 legend. Cell lines were derived as described in Fig. 2 legend. Lysates from the cell line EJ/Gpt-3 (lanes 1, 3) and from the cell line EC/Gpt-1 (lanes 2, 4) were precipitated with the monoclonal anti-p21 antiserum Y13-238 (lanes 1, 2) or with non-immune serum (lanes 3, 4). Schematic diagrams (lane 5, EJ/Gpt-3; lane 6, EC/Gpt-1) show both the relative positions of the detected p21 bands and the relationships of those bands (arrows) based on kinetic data (not shown) and previously published experiments²⁸. **b**, Analysis of the migration of p21 proteins immunoprecipitated from cells transfected with *in vitro* recombinants of the EJ oncogene (pEJ) and its homologous proto-oncogene (pEC). NIH 3T3 cells transformed with the EJ bladder tumour oncogene, its normal proto-oncogene, or recombinants between the two genes were first biologically cloned in 0.35% agar and then metabolically labelled with ^{35}S -methionine for 18 h. Lysates were prepared and immunoprecipitated (5×10^6 c.p.m. of TCA-precipitable counts) by a monoclonal antibody that detects the p21 encoded by Ha-MuSV but not the p21 encoded by Ki-MuSV (Y13-172)^{25,32}. Dissolved immunoprecipitates were then resolved by electrophoresis in a 12% SDS-polyacrylamide gel. Lanes 1a-7a, no antibody; lanes 1b-7b, anti-Harvey p21 monoclonal antibody. Cell lysates were from: NIH 3T3 cells (lane 1); cells transformed with the proto-oncogene (the 3-kb *SacI* fragment of the pEC proto-oncogene that has been activated by fusion with a retrovirus LTR transcriptional promoter described in ref. 35) (lane 2); cells transformed with the EJ oncogene (6.6-kb fragment in pBR) (lane 3); clone 511-74, transformed with the ligation of proto-oncogene 1-kb *SacI* fragment to EJ oncogene 3-kb *SacI* fragment (construction b2, Fig. 5) (lane 4); cells transformed with ligations of a fragment of the EJ oncogene extending from the *XhoI* site to the second *BstEII* site to a clone of the proto-oncogene from which the homologous fragment had been removed (construction e2, Fig. 5) (lanes 5 and 6); and cells transformed with the ligation of the EJ oncogene to the left of the *KpnI* site to the proto-oncogene to the right of this site (construction a2, Fig. 5) (lane 7). **c**, Analysis of the migration of the p21 proteins immunoprecipitated from cells transfected with the *in vitro* recombinant in which the 350-bp *XmaI-KpnI* fragment of the pEJ oncogene clone was inserted into the corresponding region of the pEC proto-oncogene clone. Cells were metabolically labelled with ^{35}S -methionine for 3 h; cell lysates were prepared, immunoprecipitated and analysed as described in Fig. 1 legend. Cell lines EC/Gpt-1 and EJ/Gpt-3 were derived as described in Fig. 2 legend. The *in vitro* recombinant was constructed as described in Fig. 5 legend, part h2. The resulting construct was transfected as described in Fig. 2 legend and a transformed focus was picked and expanded. Immunoprecipitations were performed with non-immune serum (lanes 1-3) or with the monoclonal antiserum (Y13-238) which precipitates the Ha-MuSV p21 (lanes 4-6). Cell lysates were prepared from EJ/Gpt-3 (lanes 1 and 4); EC/Gpt-1 (lanes 2 and 5); and the *XmaI-KpnI* recombinant transfected cell line (lanes 3 and 6).

Fig. 5 Transfection data and structures of *in vitro* genetic recombinants between the molecular clones of the EJ transforming gene (pEJ) and its normal cellular homologue (pEC). The restriction map shows the cleavage sites for various enzymes within the 6.6-kb *Bam*HI insert in pBR322. All sites specific for the enzymes are shown except for *Xma*I which cuts in several other places which have not been well characterized. The site shown is the only *Xma*I site between the first *Bst*EII site and the *Kpn*I site. The solid boxes on the map show the locations of coding exons. pEJ/pEC chimaeras are shown, with segments derived from pEJ shown as solid bars and segments from pEC shown as open bars. pEJ and pEC were cleaved with the indicated enzymes either to completion or in a partial digest as required to obtain each indicated fragment. The products were separated by electrophoresis through 1.2% agarose and eluted by melting in NaI and adsorbing to glass beads. The fragment containing pBR322 was then treated with calf intestinal phosphatase. The indicated fragments were joined either with the enzyme T4 DNA ligase or in a mock ligation without enzyme. Constructs a-e were made in bimolecular ligations. Constructs in f were made by mixing the three fragments simultaneously and in g and h by mixing the four fragments simultaneously. The ligation mixtures were directly transformed into the HB101 strain of *Escherichia coli*. Only when colonies from mock ligations were less than 2% of the ligations were colonies analysed for the presence of clones with appropriate restriction maps; 20 ng of each clone were transfected to NIH 3T3 cells as described in Fig. 2 legend and then carried without selection until foci were visualized in 10-14 days. Results of the transfections are shown in the first column. The second column shows the number of independent bacterial colonies screened and then transfected into NIH 3T3 cells.



done in three ways. In the simpler constructions, involving ligations of two fragments at a time, we verified results obtained with amplified recombinant clones by directly transfecting the unamplified products of ligation reactions and of mock ligations containing isolated fragments not treated with the ligase. A second confirmation depended on the fact that the plasmids pEJ and pEC contained their respective cellular genes inserted in the pBR322 plasmid vector in opposite orientations. Thus, we could learn the origin of one parent of a recombinant by diagnostic restriction digests of the flanking plasmid regions. Since contaminating pEC could itself not give a false positive result (as it is not transforming), any active clone carrying proto-oncogene flanking sequences must have arisen as a consequence of acquisition of portions of the transforming gene. Finally, we confirmed all of our results with several independent clones obtained from a ligation reaction. The products of a ligation were used only if mock ligation of the same fragments gave no more than 2% of the bacterial colonies seen on use of the DNA ligase.

The results of transfecting the recombinant clones are summarized in Fig. 5. As can be seen, we ultimately identified a genetic region 350 nucleotides long which, when transferred from the oncogene to a corresponding region in the proto-oncogene, is able to impart activity to the latter. This region extends from the first *Xma*I endonuclease site to the *Kpn*I site (Fig. 5); 55% of this region consists of the first coding exon, 10% is 5' to the coding region and 35% is part of the first intron.

Detection of differences in size of the gene product from the *in vitro* recombinants

In experiments described above, we had identified a difference in the migration of the p21 protein encoded by the oncogene and the proto-oncogene. Having now determined a short region of the gene which contained the transforming lesion, it became important to ascertain whether the region also contains the specificity for the altered protein size. We therefore carried out immunoprecipitations on cells transfected with the products of the *in vitro* recombinants. As seen in Fig. 4b, the protein brought down in lysates from *Xho*I-second *Bst*EII site, *Sac*I-*Sac*I, and

*Xho*I-*Kpn*I recombination transfectants all co-migrate with the EJ protein and have a mobility which differs from that of the EC protein. Similarly, Fig. 4c demonstrates this result for the *Xma*I-*Kpn*I recombinants. The lysates analysed in panel 4b were from cultures that were labelled for 18 h, resulting in high levels of label in the lower molecular weight forms of the p21, and undetectable amounts of label in the kinetically unstable higher molecular weight forms. We conclude that the phenotypes of oncogenic transformation and altered electrophoretic migration co-segregate, and the two are encoded by the same 350-bp segment of DNA. As indicated below, the altered migration rate is probably a reflection of a functionally important alteration of the protein.

Nucleotide sequence of the transforming lesion

Having identified a short fragment of biological significance, we then sequenced this region of the oncogene and the proto-oncogene. Figure 6 compares the sequences of the two genes in this segment obtained by sequencing both strands. The DNA sequence described in the figure was obtained by the forward and backward dideoxy DNA sequencing technique of Seif *et al.*²⁹ and by the chemical procedure of Maxam and Gilbert³⁰.

The difference between the two segments is in the p21 encoding region of the first known exon, specifically 60 nucleotides from the *Xma*I cleavage site. It occurs in a triplet that encodes glycine in the normal rat (M. Ruta *et al.*, unpublished data) and human c-Ha-ras genes. The sequence observed in the EJ oncogene causes conversion to a valine. As we discuss below, we believe that this alteration is responsible for the alteration in function of the p21 protein, and for the oncogenic activation of the c-Ha-ras gene that occurred in the EJ bladder carcinoma.

Alteration of a diagnostic restriction endonuclease cleavage site

A second independent consequence of the single base change was the alteration in the cleavage sites of two different site-specific endonucleases. The sequence GCCGGC occurs in the

proto-oncogene, and thus represents the recognition site of the endonuclease *NaeI*. It also contains the CCGG recognition site of the endonuclease *HpaI*. Both of these are changed in the oncogene, the equivalent sequence of which reads GCCGTC. The *NaeI* endonuclease is the more useful of the two since it cleaves DNA less frequently, and we used it to independently verify the two sequences. As expected, the pEC clone exhibited one more cleavage site in its inserts than its pEJ counterpart (data not shown). Perhaps more useful was the retrospective verification of the *in vitro* recombinant clones. The allele specifying transformation and abnormal p21 migration was seen to precisely co-segregate with the allele disallowing *NaeI* cleavage at this site (data not shown).

Discussion

Use of gene transfer and molecular cloning had previously made it possible to localize an important lesion of the EJ bladder carcinoma genome to a 6.6-kb DNA segment constituting only 10^{-6} of the genetic content of the tumour cell. The segment appears to contain the entire transforming activity previously associated with the whole tumour cell DNA. This oncogene is an important, but perhaps not the sole, determinant of the transformed phenotype of these carcinoma cells. Sequence hybridization showed that this oncogene was related in structure to a similar sequence existing in the normal cellular genome¹²⁻¹⁵. It appeared that the oncogene arose from the activation of these normal sequences, and that this activation occurred during the process of carcinogenesis which led to formation of the original EJ carcinoma. Experiments described in this report were designed to resolve and define the critical differences that distinguish the oncogene from its proto-oncogene precursor.

It was apparent that a definition of these differences could not be achieved by simple comparison of the sequences of the two genes. As the oncogene and its normal proto-oncogene counterpart sequence were derived from the DNAs of separate individuals, most sequence differences might reflect naturally occurring, silent polymorphisms at this locus. Other sequence differences could be consequences of mutational insults suffered during carcinogenesis, which were silent functionally and thus of no importance to the activation process. These considerations caused us to undertake the *in vitro* recombination experiments in order to narrow the area of functional importance to a segment so small that it would probably contain few if any silent mutations. Only after narrowing the functional segment to 350 bp did we initiate DNA sequence studies.

The sequence difference that we observed in this region could have only one consequence for the structure of the p21 protein—a simple amino acid substitution leading to the replacement of a glycine by a valine residue. This substitution appears to represent the critical agent of the conversion of the proto-oncogene into an active oncogene.

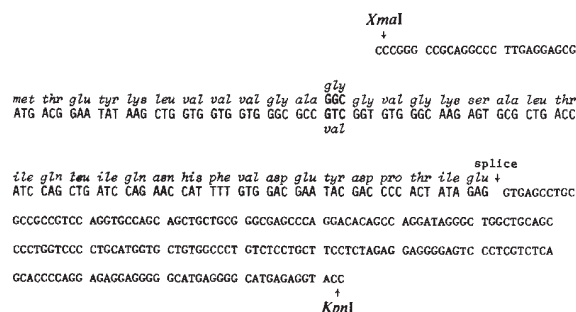


Fig. 6 Comparison of the DNA sequence of the molecular clone of the EJ transforming gene (pEJ) and its non-transforming cellular homologue (pEC). Sequence data between the restriction enzyme sites *XmaI* and *KpnI* are shown (see Fig. 5). The coding DNA strand is printed as is the inferred amino acid sequence. For coding regions where pEJ and pEC differ, both sequences are indicated. Sequences were determined by the forward and backward dideoxy DNA sequencing technique of Seif *et al.*²⁹ and by the chemical procedure of Maxam and Gilbert³⁰.

Such a simple substitution might not be expected to effect a profound change in the function of the p21 protein. However, several considerations seem to confer importance on the structural alteration. The first stems from comparison of this domain of p21 encoded by the human c-Ha-ras-1 gene, the rat c-Ha-ras-1 gene (Ruta *et al.*, unpublished results) and the v-Ha-ras oncogene of Harvey sarcoma virus³¹. The 37-residue long amino acid sequences encoded by the first exons of the two cellular genes are identical, indicating strict evolutionary conservation of this region. However, analysis of the Harvey sarcoma virus oncogene reveals that it deviates from its direct rat cellular antecedent in only one position in this domain, a glycine to arginine conversion at precisely the same residue that is altered in the EJ oncogene. Thus, we speculate that alteration of this critical residue was important both in the activation of the v-Ha-ras gene from its rat cellular precursor, and in the activation of the EJ bladder oncogene from its normal human counterpart. Moreover, a similar alteration may have been significant in the oncogenic activation of another member of the *ras* gene family, the v-Ki-ras gene of Kirsten murine sarcoma virus. The Kirsten transforming gene is closely related to the v-Ha-ras gene³². The only difference between the p21 of v-Ki-ras and that of v-Ha-ras in the first 36 amino acids is at position 12, where the residue in Kirsten is serine³³, again at precisely the same site as altered in the EJ and Harvey sarcoma virus encoded p21s. While sequence information is not available on the cellular homologue of v-Ki-ras, we speculate that a conversion from a glycine to a new amino acid residue at position 12 may also have been involved in the activation of this *ras* oncogene.

A second consideration stems from examination of the specific amino acid changes observed in these cases. In both instances, glycine is replaced by an amino acid having a relatively bulky side chain. Glycine is an anomaly among the 20 amino acids, because it lacks a side chain. Thus, it is able to participate in extremes of bending and folding of the polypeptide backbone and is the strongest breaker of α -helices³⁴. Thus, replacements of glycine by valine or arginine represent abrupt changes in the local stereochemistry of a protein.

We speculate that the loss of glycine at residue 12 causes a significant change in an essential domain of the p21 protein. One consequence of this change may be a conformational shift of the protein, leading in turn to the aberrant electrophoretic migration or processing of the p21 proteins. A second, more important consequence is a profound effect on the function of the p21 protein. This alteration probably affects interaction of the p21 with cellular targets. A precedent exists for other single amino acid changes having profound effects on cellular and organismic physiology. The most well known of these is the sickle-cell syndrome, in which a glutamine to valine conversion affects the solubility of haemoglobin within erythrocytes.

The importance we attach to alteration of protein structure would seem to contradict a series of experiments of recent years that indicate up-regulation as the pivotal event in carcinogenesis. These experiments include the activation of the *myc* proto-oncogene occurring during leukaemogenesis of avian retroviruses^{35,36}; and the demonstration that *in vitro* fusion of a retroviral long terminal repeat (LTR) promoter and a cellular proto-oncogene results in an actively transforming gene^{20,37,38}. These latter experiments are particularly germane, since some of them demonstrate activation of clones of the rat and human c-Ha-ras proto-oncogenes^{20,38}. It is unlikely that the protein-encoding sequences of these c-Ha-ras genes have undergone any structural changes during construction of these viral-cellular chimaeras. Rather, it appears that the only essential difference between the proto-oncogenes and their LTR-activated counterparts lies in rates of expression. This means that the *ras* proto-oncogene can be activated by a second independent mechanism, in principle as effective in creating an oncogene as the one described here.

We point out that oncogenes of other tumours have also been traced to *ras* genes. Specifically, colon and lung carcinomas

(ref. 15 and M. McCoy, J. J. Toole, E.H.C. and R.A.W., in preparation) as well as fibroblastic tumours (L. Parada and R.A.W., unpublished results) have been found to carry oncogenes derived from activation of cellular *Ki-ras* genes. As discussed above, the N-terminal regions of the *Ha-ras* and *Ki-ras* proteins are extremely similar, and the *Ki-ras* proteins of many of these tumours also exhibit aberrant electrophoretic migration rates (manuscript in preparation). We predict that activation of many of these other oncogenes will depend on structural alterations very similar to those reported here.

Most amino acid sequence alterations are either neutral or deleterious to protein function. Few are able to actively potentiate the normal functions of a protein. We suggest that only a small number of sites on the p21 protein can be altered in a fashion leading to oncogenic activation. Most mutations will affect other residues whose alteration will be unproductive for oncogenic conversion. The target for oncogenic conversion may therefore be exceedingly small, and may even be confined to the Gly-12 codon. In such a case, the restriction site for *NaeI*, which totally spans that codon, may provide an ideal diagnostic tool for detecting a critical change in the genome.

The present data suggest that the alteration of one nucleotide

in one bladder cell leads to the creation of an activated oncogene. There are three possible point mutations at this position, and it is perhaps not coincidental that the G-T transversion observed here is precisely that mutation favoured by many suspected bladder carcinogens³⁹. The oncogene resulting from this mutation was probably an important determinant in the subsequent outgrowth of a lethal neoplasm. The point mutation implicated as a central event in this oncogenic transformation represents the first demonstration of a lesion in cellular DNA whose occurrence is directly related to the carcinogenic process.

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- McCann, J., Choi, E., Yamasaki, E. & Ames, B. N. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5135-5139 (1975).
- McCann, J. & Ames, B. N. *Proc. natn. Acad. Sci. U.S.A.* **73**, 950-954 (1976).
- Bridges, B. A. *Nature* **261**, 195-200 (1976).
- Bouck, N. & diMayorca, G. *Nature* **264**, 722-727 (1976).
- Shih, C., Shilo, B., Goldfarb, M. P., Dannenberg, A. & Weinberg, R. A. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5714-5718 (1979).
- Cooper, G. M., Okenquist, S. & Silverman, L. *Nature* **284**, 418-421 (1980).
- Shih, C., Padhy, L. C., Murray, M. J. & Weinberg, R. A. *Nature* **290**, 261-264 (1981).
- Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1181-1184 (1981).
- Perucho, M. *et al. Cell* **27**, 467-476 (1981).
- Lane, M. A., Sainten, A. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5185-5189 (1981).
- Shilo, B. & Weinberg, R. A. *Nature* **289**, 607-609 (1981).
- Goldfarb, M., Shimizu, K., Perucho, M. & Wigler, M. *Nature* **296**, 404-409 (1982).
- Shih, C. & Weinberg, R. A. *Cell* **29**, 161-169 (1982).
- Pulciani, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2845-2849 (1982).
- Der, C., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3637-3640 (1982).
- Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* **297**, 474-479 (1982).
- Santos, E. *et al. Nature* **298**, 343-347 (1982).
- Scolnick, E. M. & Parks, W. P. *J. Virol.* **13**, 1211-1219 (1974).
- Shih, T. Y. *et al. J. Virol.* **27**, 45-55 (1978).
- DeFeo, D. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 3328-3332 (1981).
- Chang, E. H., Gonda, M. A., Ellis, R. W., Scolnick, E. M. & Lowy, D. R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4848-4852 (1982).

- Ellis, R. W. *et al. J. Virol.* **36**, 408-420 (1980).
- Wu, Y.-J., *et al. Cell* (submitted).
- Ellis, R. W., DeFeo, D., Furth, M. E. & Scolnick, E. M. *Cell* (in the press).
- Furth, M. E., Davis, L. J., Fleurdelys, B. & Scolnick, E. M. *J. Virol.* **43**, 294-304 (1982).
- Mulligan, R. & Berg, P. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2072-2076 (1981).
- Wigler, M. *et al. Cell* **16**, 777-785 (1979).
- Shih, T. Y. *et al. J. Virol.* **42**, 253-261 (1982).
- Seif, I., Khoury, G. & Dhar, R. *Nucleic Acids Res.* **8**, 2225-2238 (1980).
- Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560-564 (1977).
- Dhar, R. *et al. Science* **217**, 934-936 (1982).
- Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. *Virology* **96**, 64-79 (1979).
- Tsuchida, N., Ryder, T. & Ohtsubo, E. *Science* **217**, 937-939 (1982).
- Cantor, C. R. & Schimmel, P. R. in *Biophysical Chemistry* Vol. 1, 303-304 (Freeman, San Francisco, 1980).
- Neel, B. G., Hayward, W. S., Robinson, H. L., Fang, J. & Astrin, S. M. *Cell* **23**, 323-334 (1981).
- Payne, G. S. *et al. Cell* **23**, 311-322 (1981).
- Blair, D. G. *et al. Science* **212**, 941-943 (1981).
- Chang, E. J., Furth, M. E., Scolnick, E. M. & Lowy, D. R. *Nature* **297**, 479-483 (1982).
- Swenson, D. H. & Kadlubar, F. F. in *Microbial Testers Probing for Carcinogenesis* (ed. Felkner, I. C.) 3-33 (Dekker, New York, 1981).
- Varmus, H. E., Quintrell, N. & Ortiz, S. *Cell* **25**, 23-36 (1981).
- Rigby, P. W. *et al. J. molec. Biol.* **13**, 237-251 (1977).
- Wahl, G. M., Stern, M. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3683-3687 (1979).
- Manley, J. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 3855-3859 (1980).
- Graham, F. L. & van der Eb, A. J. *Virology* **52**, 456-471 (1973).
- Andersson, P., Goldfarb, M. P. & Weinberg, R. A. *Cell* **16**, 63-75 (1979).

A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene

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The genetic change that leads to the activation of the oncogene in T24 human bladder carcinoma cells is shown to be a single point mutation of guanosine into thymidine. This substitution results in the incorporation of valine instead of glycine as the twelfth amino acid residue of the T24 oncogene-encoded p21 protein. Thus, a single amino acid substitution appears to be sufficient to confer transforming properties on the gene product of the T24 human bladder carcinoma oncogene.

DNA-MEDIATED gene transfer techniques have made it possible to identify the presence of dominant transforming genes (oncogenes) in a variety of human tumours (for review see ref. 1). Although only a small number of human tumour DNAs have been shown to be capable of transforming normal cells in transfection assays, oncogenes have been detected in tumours representative of each of the major forms of human cancer²⁻⁸. To date, more than 10 different human oncogenes have been

identified. One of them, present in T24 and EJ bladder carcinoma cell lines, has been isolated by molecular cloning techniques⁸⁻¹⁰. Preliminary characterization of this oncogene has revealed that it is small (less than 4.6 kilobase, kb), and that it has not undergone major genetic rearrangements⁹⁻¹¹. Comparative analysis of this bladder carcinoma oncogene with retroviral transforming (*onc*) genes has revealed that an internal fragment of the T24 oncogene is closely related to the *onc* genes of

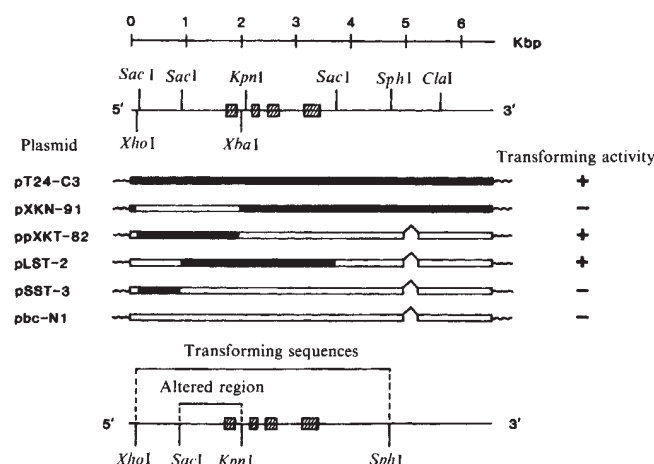


Fig. 1 Location of the region of the *c-has/bas-1* gene that underwent the genetic alterations that led to the activation of the T24 oncogene. Hybrid plasmids containing sequences derived from pT24-C3 (■) and pbC-N1 (□) were tested for their biological activity. pT24-C3, pXKT-82 and pLST-2 transformed NIH 3T3 cells in transfection assays⁸, with specific transforming activities of 5×10^4 , 1×10^4 and 0.6×10^4 ffu pmol⁻¹, respectively. In contrast, pbC-N1, pXKN-91 and pSST-3 exhibited no transforming activity. The hatched boxes located within the restricted endonuclease map shown in the top of the figure represent the predicted exons of the T24 oncogene and its normal homologue, the *c-has/bas-1* gene. Deletions (Δ), and plasmid sequences (—), are also indicated in the diagram. See text for definition of the regions encompassing the transforming sequences and the activated domain of the T24 oncogene.

Harvey and BALB murine sarcoma viruses (designated *v-has* and *v-bas*, respectively)¹¹⁻¹³. Characterization of human DNA sequences homologous to *v-has* and *v-bas* has demonstrated that the normal *c-has/c-bas-1* gene is in fact an allele of the T24 oncogene¹¹⁻¹⁴. Moreover, we found that these genes, despite their different biological properties, were indistinguishable by both heteroduplex and restriction enzyme analysis¹¹⁻¹³. In an effort to understand the molecular events involved in human

carcinogenesis, studies were undertaken to determine the number and nature of the genetic alterations that have resulted in the activation of the T24 human bladder carcinoma oncogene.

A domain within the T24 oncogene responsible for its activation

Previously, we have reported that a plasmid (pT24-C3) known to contain a 6.6-kb *Bam*HI fragment of T24 cellular DNA was capable of transforming NIH 3T3 mouse cells with a specific activity of 5×10^4 focus-forming units (ffu) per pmol. A restriction endonuclease map of this DNA fragment is depicted in Fig. 1. The transforming activity of this molecule was localized to a region within the 4.6-kb *Xho*I-*Sph*I DNA fragment based on the observations that cleavage with these two restriction endonucleases did not affect the transforming activity of pT24-C3 (ref. 11). To establish the region of the normal human *c-has/bas-1* gene that underwent the genetic alterations leading to its malignant activation in T24 bladder carcinoma cells, we constructed a series of plasmids in which fragments of the T24 transforming gene were substituted with the homologous normal sequences of *c-has/bas-1*. In reciprocal experiments, regions of the normal *c-has/bas-1* gene were replaced by the corresponding domains of its transforming allele. Figure 1 summarizes the plasmids constructed in the present studies as well as their respective biological activities. Substitution in pT24-C3 of a DNA fragment located between the *Xho*I and *Kpn*I cleavage sites by normal sequences, completely abolished its transforming activity. In contrast, replacement of the normal *Xho*I-*Kpn*I region of pbC-N1, a plasmid that contains the 6.4-kb *Bam*HI DNA clone of the normal human *c-has/bas-1* gene, by the corresponding domain of pT24-C3, generated a plasmid (pXKT-82) that transformed NIH 3T3 mouse cells with an efficiency of 10^4 ffu pmol⁻¹.

Two additional plasmids were generated by replacing the internal 0.7-kb and 3.0-kb *Sac*I DNA fragments of pbC-N1 by the corresponding regions of the T24 oncogene. As shown in Fig. 1, only the latter plasmid (pLST-2) was able to induce malignant transformation on transfection to NIH 3T3 cells. These results, taken together, establish that the genetic alterations that activated the T24 oncogene are located within the 0.9-kb *Sac*I-*Kpn*I DNA fragment of pT24-C3 DNA.

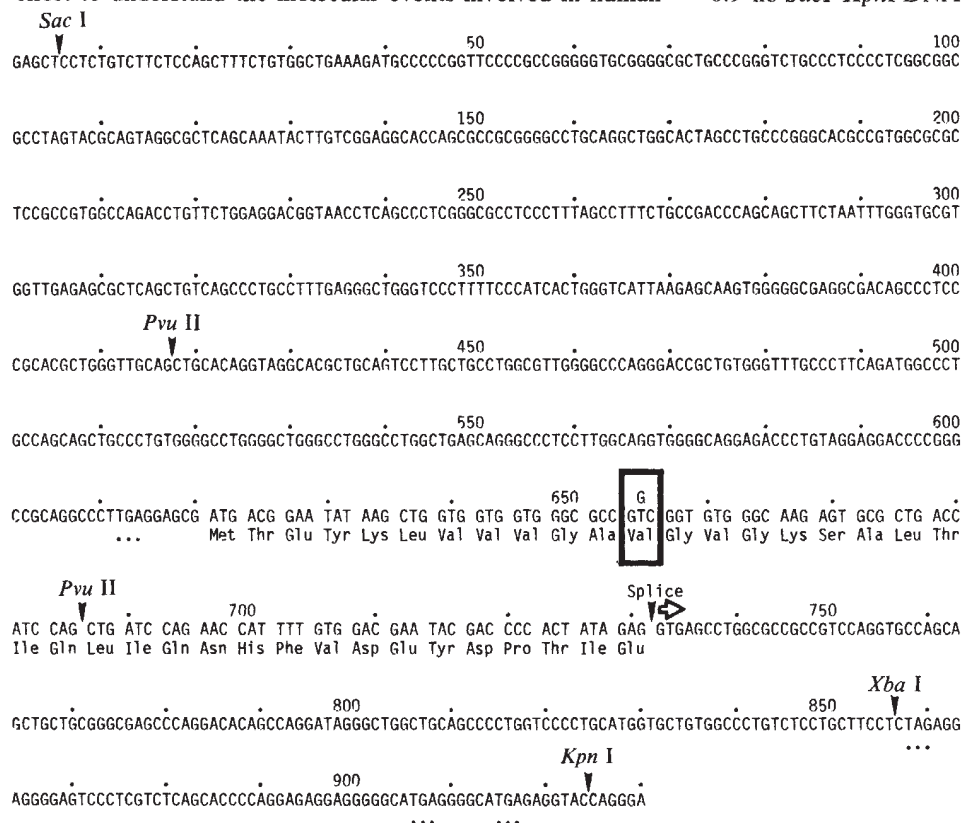


Fig. 2 Comparative sequence analysis of the 0.93-kb *Sac*I-*Kpn*I DNA fragment of the T24 oncogene and its normal human homologue *c-has/bas-1*. The upper line shows the sequence of the T24 oncogene proceeding in the 5' to 3' direction with respect to the polarity of the closely related *v-has* and *v-bas* retroviral mRNAs. The corresponding sequence of the 0.93-kb *Sac*I-*Kpn*I DNA fragment of *c-has/bas-1* has also been determined and found to be identical to that of the T24 oncogene with the exception of the nucleotide located at position 653, where we detected a G instead of a T. These nucleotide chains result in the incorporation of glycine, instead of valine, at position 12 in the p21 protein coded for by the *c-has/c-bas-1* gene. Termination codons are indicated (...). The nucleotide sequence analysis was performed according to the procedures of Maxam and Gilbert²³.

Fig. 3 Predicted amino acid sequence of the first exon of the normal human *c-has/bas-1* gene and its transforming allele, the T24 oncogene. Comparison with the first 37 amino acids predicted for the p21 proteins coded for by Harvey-MSV¹⁵ and Kirsten-MSV¹⁸. The letter code for the amino acids is: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; and Y, Tyr.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Human <i>c-bas/ras^H</i> :	M	T	E	Y	K	L	V	V	V	G	A	G	G	V	G	K	S	A	L	T	I	Q	L	I	Q	N	H	F	V	D	E	Y	D	P	T	I	E
T24 oncogene :	M	T	E	Y	K	L	V	V	V	G	A	V	G	V	G	K	S	A	L	T	I	Q	L	I	Q	N	H	F	V	D	E	Y	D	P	T	I	E
Harvey-MSV :	M	T	E	Y	K	L	V	V	V	G	A	R	G	V	G	K	S	A	L	T	I	Q	L	I	Q	N	H	F	V	D	E	Y	D	P	T	I	E
Kirsten-MSV :	M	T	E	Y	K	L	V	V	V	G	A	S	G	V	G	K	S	A	L	T	I	Q	L	I	Q	N	H	F	V	D	E	Y	D	P	T	I	Q

Comparative sequence analysis of the 0.9-kb *SacI-KpnI* DNA fragments of pT24-C3 and pbc-N1

The nucleotide sequence of the 0.9-kb *SacI-KpnI* DNA fragments of the T24 oncogene and its normal homologue was next established. To our surprise there was only a single (nucleotide) change, located at position 655 from the *SacI* cleavage site. Figure 2 shows that the guanosine residue present in the normal *c-has/bas-1* gene has been changed into a thymidine residue in the T24 oncogene. Thus the activation of the *c-bas/ras-1* gene in T24 human bladder carcinoma cells was caused by a single point mutation.

The mutated guanosine residue was found to be part of the hexanucleotide sequence GCCGGC, which is specifically recognized by the restriction endonuclease *NaeI*. Thus, we used this enzyme to verify the results obtained by nucleotide sequence analysis. We observed that *NaeI* cleaved the 6.4-kb *BamHI* insert of pbc-N1 five times, yielding four internal DNA fragments, of sizes 0.2, 0.4, 0.6 and 0.9 kb. In contrast, digestion of pT24-C3 with *NaeI* yielded only three internal fragments, of sizes 0.2, 0.6 and 1.3 kb (data not shown). The loss of the *NaeI* cleavage site within the 1.3-kb internal fragment confirms the nucleotide sequence shown in Fig. 2.

Heteroduplex analysis of the T24 oncogene and its normal allele with *v-has* and *v-bas* had indicated that the putative coding sequences of the human genes are distributed among four exons, of which only the first one lies within the 0.9-kb *SacI-KpnI* fragment^{11,14}. We have sequenced the entire coding region of the T24 oncogene and identified the four exons by comparison of these sequences with those of *v-has*¹⁵ and *v-bas*¹⁶. This sequence analysis demonstrated that the first exon ends at position 731 from the *SacI* cleavage site (Fig. 2). At this position, we detected the consensus donor splice sequence AGAGG¹⁷. Thus, in agreement with previous heteroduplex analysis and restriction enzyme data^{11,14}, the first exon of the T24 oncogene contained the coding information for 37 amino acids extending from position 621 to 731. These observations located the mutated G residue at the second base of a triplet coding for glycine, the predicted twelfth amino acid residue of the normal human p21 protein. Substitution of this guanosine residue for thymidine results in the change of the glycine-coding triplet GGC to GTC, which codes for valine. Thus, a single amino acid substitution (Gly→Val) seems to be sufficient to confer transforming properties to the gene product of the T24 human bladder carcinoma oncogene.

A common genetic alteration in human and retroviral transforming genes

The amino acid sequences of Harvey and BALB-MSV-encoded p21 proteins have been identified by nucleotide sequence analysis of their corresponding *onc* genes, *v-has* and *v-bas*, respectively^{15,16}. Comparison of the first 37 amino acids of the p21 protein coded for by the normal *c-has/bas-1* human gene with their retroviral counterparts shows complete identity except at position 12, the same amino acid residue that is altered in the T24 oncogene (Fig. 3). Whereas arginine is present in Harvey-MSV p21¹⁵, lysine was found in BALB-MSV p21¹⁶. Interestingly, the normal rat homologue of human *c-has/bas-1*, the gene that presumably recombined with Moloney-MuLV to generate Harvey-MSV, also possesses glycine at position 12 (M. Ruta, personal communication). These results suggest that

mutations affecting the coding properties of the twelfth codon of normal *c-has/bas-1* genes may be sufficient for the acquisition of malignant properties.

Recently, Tsuchida *et al.* have also reported the nucleotide sequence of *v-kis*, the *onc* gene of Kirsten-MSV¹⁸. When the first 37 amino acids of the deduced sequence of Kirsten p21 were compared with those of *c-has/bas-1*, only two changes were detected: Gly→Ser in position 12, and Glu→Gln in position 37. Thus, it is possible that changes in the glycine residue at position 12 may have also activated *c-kis* genes. This hypothesis would predict that another human transforming oncogene, present in a large variety of human carcinomas and known to contain the human *c-kis-2* gene,^{5,6,8,12,14,19} has been activated by the same mechanism that originated the T24 oncogene.

Activation of the human *c-has/bas-1* gene: a qualitative or quantitative mechanism?

That each of the known p21 transforming proteins has a different amino acid at residue 12 suggests that their malignant properties are the result of the elimination of the glycine residue present in their normal counterparts, rather than due to a specific transition from one type of amino acid residue to another. Glycine residues are known to alter the α -helical structure of proteins. We compared the predicted secondary structures of the p21 proteins coded for by the T24 oncogene and its normal human homologue using the computerized method of Garnier *et al.*²⁰. Such an analysis revealed that the N-terminal region of the *c-has/bas-1*-encoded p21 protein contains two α -helical regions of 10 and 13 amino acids joined by a hinge region of 7 amino acids that provides flexibility for folding. When the glycine at position 12 is replaced by valine in the transforming p21 protein, the size of the hinge region decreases to a stretch of two amino acids, resulting in an increased helical content of the N-terminal region of this protein. This change should result in the prominent projection of the N-terminal domain of transforming p21 proteins away from the central core of the molecule.

The biological significance of the structural alteration of human and retroviral p21 transforming proteins remains to be determined. Shih *et al.* have recently reported that the Harvey-MSV p21 protein is processed before its association with membranous structures²¹. Whether the presence of a glycine residue in position 12 confers on the normal cellular p21 protein a configuration that affects either its processing or its association with putative cellular targets remains to be determined. Whatever the mechanism might be, our findings indicate that the difference between the normal *c-has/bas-1* and the T24 oncogene-encoded p21 proteins is of a qualitative nature.

However, it is also possible that quantitative alterations in the expression of the normal p21 protein induce malignant transformation. Chang *et al.* have shown that the normal *c-has/bas-1* can transform NIH 3T3 mouse cells when this gene comes under the regulatory influence of a retroviral LTR²². In this case high levels of normal p21 protein can be detected in the transformed cells. Thus it is possible that increasing amounts of normal p21 protein may overcome the structural limitations imposed by the presence of a glycine residue at position 12. However, the possibility that over-expression of the normal *c-has/bas-1* genes may induce morphological transformation by a different mechanism than that of their transforming counterparts cannot be ruled out at present.

Implications

The present studies have located the genetic changes leading to the activation of the T24 bladder carcinoma oncogene within a 0.9-kb *SacI-KpnI* DNA fragment of the human *c-has/bas-1* gene. This region contains some 5' putative regulatory sequences, as well as the first exon (Fig. 2). Comparative sequence analysis of these 0.9-kb DNA fragments derived from the T24 oncogene and its normal counterpart revealed a single base pair difference: a point mutation (G→T) within the exon sequences that led to the substitution of a glycine residue in the normal *c-has/bas-1* gene-encoded p21 protein to a valine residue in the corresponding translational product of the T24 oncogene. These results, taken together, indicate that a single point mutation was responsible for the activation of this oncogene in T24 human bladder carcinoma cells.

These findings may have important practical applications. The mutated guanosine residue is part of a sequence (GCCGGC) recognized by the restriction endonucleases *NaeI* (GCCGGC), *HpaII* (CCGG) and *MspI* (CCGG). The G→T change observed in the T24 oncogene eliminated the cleavage site for these three enzymes. Neither *NaeI* nor *HpaII* appear to cleave this sequence in human DNA, probably due to DNA methylation. However it is possible that *MspI*, which recognizes

the CCGG sequence whether methylated or not, could be used for the screening of human DNAs for the presence of T24-like oncogenes. Similarly, antibodies capable of specifically recognizing the altered domain of transforming p21 proteins may also be used to detect activated *c-has/bas* genes.

Demonstration that a single genetic change can activate a human transforming gene poses the dilemma as to how to relate these observations with the widely accepted multi-stage model for the development of human neoplasias. It is possible that activation of the T24 oncogenes may represent a late, irreversible step in oncogenesis. Support for this hypothesis comes from the fact that NIH 3T3 mouse cells, the cells used to identify this oncogene, are thought to be pre-neoplastic rather than normal cells. However, the possibility that this oncogene may have a role in the onset of certain human cancers cannot be excluded at present.

Development of biochemical and immunological reagents capable of specifically detecting the presence of the T24 bladder carcinoma oncogene (or its gene product) in both naturally and experimentally induced neoplasms should help to define the role of this dominant transforming gene in carcinogenesis.

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- Cooper, G. M. *Science* **217**, 801–806 (1982).
- Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1181–1184 (1981).
- Shih, C., Padhy, C., Murray, M. & Weinberg, R. A. *Nature* **290**, 261–264 (1981).
- Lane, M. A., Saiten, A. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5185–5189 (1981).
- Murray, M. J. *et al. Cell* **25**, 355–361 (1981).
- Perucho, M. *et al. Cell* **27**, 467–476 (1981).
- Lane, M. A., Saiten, A. & Cooper, G. M. *Cell* **28**, 873–880 (1982).
- Pulciani, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2845–2849 (1982).
- Goldfarb, M., Shimizu, K., Perucho, M. & Wigler, M. *Nature* **296**, 404–409 (1982).
- Shih, C. & Weinberg, R. A. *Cell* **29**, 161–169 (1982).
- Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S. & Barbacid, M. *Nature* **298**, 343–347 (1982).
- Der, C. J., Krontiris, T. G. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3637–3640 (1982).
- Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* **297**, 474–478 (1982).
- Chang, E. H., Gonda, M. A., Ellis, R. W., Scolnick, E. M. & Lowy, D. R. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4848–4852 (1982).
- Dhar, R. *et al. Science* **217**, 934–937 (1982).
- Reddy *et al.* (in preparation).
- Sharp, P. A. *Cell* **23**, 643–646 (1981).
- Tsuchida, N., Ryder, T. & Ohtsubo, E. *Science* **217**, 937–939 (1982).
- Pulciani, S., Santos, E., Lauver, A. V., Long, L. K. & Barbacid, M. *Nature* (in the press).
- Garnier, J., Osguthorpe, D. J. & Robson, B. *J. molec. Biol.* **120**, 97–120 (1978).
- Shih, T. Y. *et al. J. Virol.* **42**, 253–261 (1982).
- Chang, E. H., Furth, M. E., Scolnick, E. M. & Lowy, D. R. *Nature* **297**, 479–483 (1982).
- Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).

LETTERS TO NATURE

UV observations of TT Arietis and the magnetic rotator hypothesis

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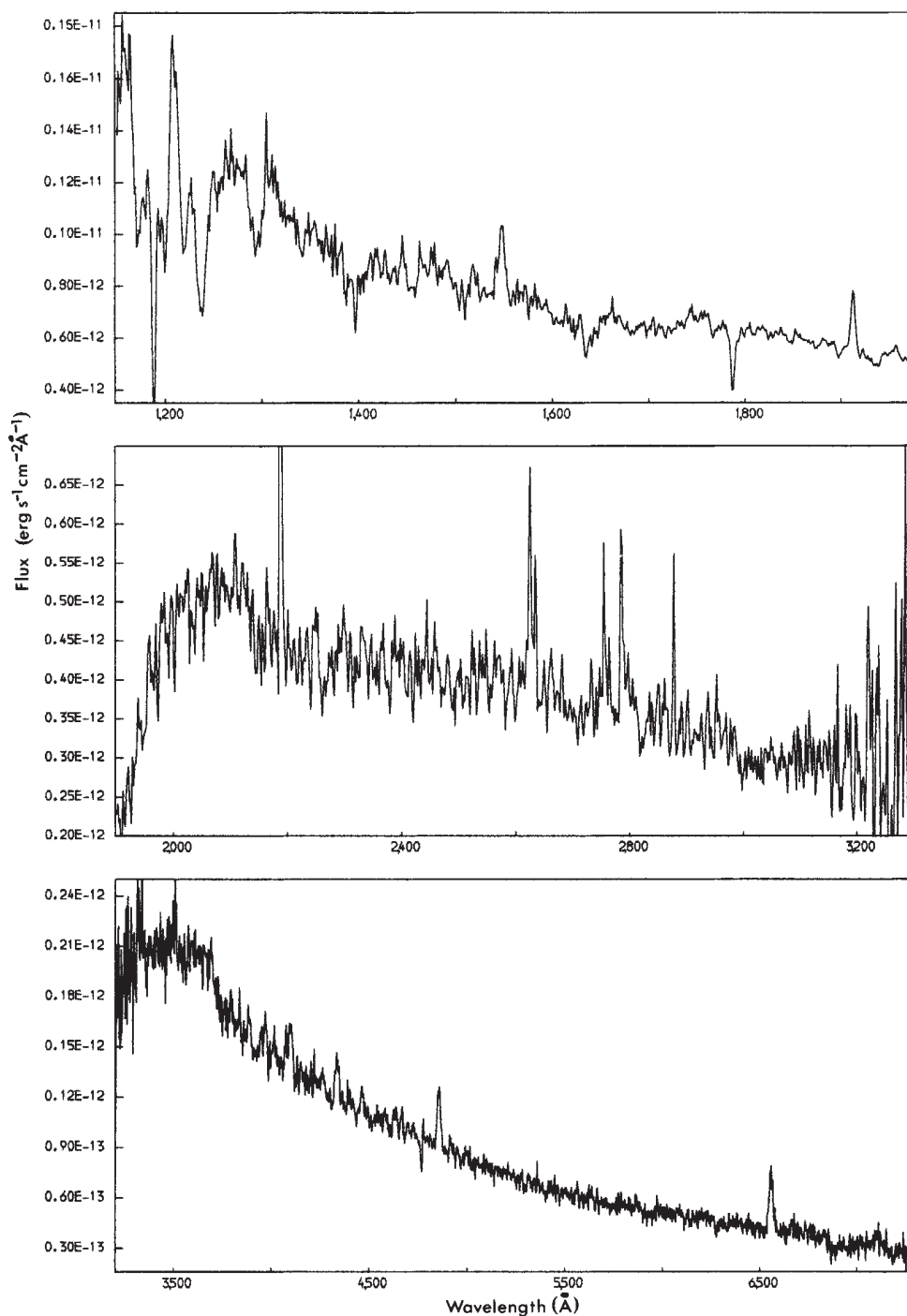
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TT Arietis, one of the brightest cataclysmic variables, $m_v \sim 10.2 \rightarrow 11.0$, is usually classed as a novalike variable, but after a recent dip in brightness to $m_v \sim 14.5$, Krautter *et al.*¹ suggested it to be a dwarf nova in an extended high state. Photometric light curves^{2–4} generally show a sinusoidal light variation with a period of 0.1329 days and no eclipses⁵. Cowley *et al.*⁶ have measured radial velocity curves and find a period of 0.13755 days. We have made⁷ IR measurements of the system from which we deduce a distance for TT Ari of 100 ± 20 pc based on a colour change for the system from V to K of $+0.4$ while the system is faint to $V-K = -0.17$ when brighter. The red colour is caused by dilution of the primary 2.2- μ m light by the light of the secondary, giving $m_K = 11.75^{+0.45}_{-0.25}$ for the secondary star; Bailey's method⁸ then leads to the distance. The sinusoidal light variations have generally been interpreted as due to a hot spot on the edge of an accretion disk. Since good quality IUE spectra can be made with exposure times of about 15 min, we decided to observe as many IUE spectra as possible round the binary period with a view to studying the behaviour of the hot spot at UV wavelengths. We now report that the variations at these and X-ray wavelengths round the orbital cycle rule out a hot spot/accretion disk model for TT Ari. Instead we propose that it has a magnetized white dwarf primary rotating with the photometric period of 0.1329 days.

Four short and four long wavelength low dispersion spectra were obtained with the IUE satellite spectrometer on 9 January, 1982. Between each exposure the fine error sensor (FES) was used to measure the white light variation of TT Ari. The spectra taken nearest to maximum light, are shown in Fig. 1, together with an optical spectrum measured by M. J. Ward with the Anglo Australian Telescope. Although the spectra appear noisy, most of the features are repeatable from one spectrum to the next. The strongest features are: Ly α 1,216 e (geocoronal?); N V 1,238 a; Si IV 1,393 a; C IV 1,549 e (2.9); He II 1,640 a; and Mg II 2795 e (2.1) where 'a' denotes absorption, 'e' emission and the number in parentheses the variation in line strength (maximum/minimum). The variation of the emission lines roughly follows that of the continuum variation. Regions as free as possible of absorption or emission lines were used to measure the continuum light variations. The continuum was averaged over a bandwidth of 50 Å. The only significant errors in the measurements are due to the photometric accuracy of the IUE cameras, namely $\approx \pm 10\%$.

Figure 2 shows the four point continuum light curves at 1,265, 1,700, 2,110, 2,510 and 3,010 Å, together with the nine point white light FES light curve. The amplitude of the white light curve corresponds to $m_v = 11.86 \rightarrow 12.36$; thus TT Ari was unusually faint. The solid curves are an attempt to fit a curve of the same shape as the white light curve but with variable amplitude through the UV points. The main difficulty in getting a good fit to the UV points is that the FES points represent ~ 1 min of integration and are thus prone to the flickering whereas the UV points result from spectra with 15-min exposure times. Whichever numbers are used for the amplitude of the variations the trend is clear; maximum amplitude being found for white light and the hard UV with a minimum variation in between at 3,010 Å.

Fig. 1 The short and long wavelength IUE spectra together with the optical spectrum of TT Ari.



The FES curve apparently shows two maxima within the photometric period of 3 h 11 min making it not immediately recognizable as a typical light curve for TT Ari. This is not surprising remembering that there are only nine points (each of ~ 1 min integration) and that TT Ari shows considerable flickering. The larger of these two maxima can, however, be identified as the usual maximum since its epoch fits with the photometric period to some optical observations carried out in November 1981. A secondary maximum is also apparent in some of the published light curves. Ideally observations should have continued for two or more periods but this was not possible since the IUE satellite was beginning to overheat in this pointing direction. Also the 20 min preparation time needed for the IUE cameras makes it impossible to improve the time resolution without resorting to 'trailed' spectra which are difficult to analyse.

The reason for this changing amplitude can be seen from

Fig. 3 which shows the five UV wavelengths together with *U*, *B* and *V* points derived from the FES counts and the known colours and changes in colours, taken from ref. 2. It is clear that the spectrum of TT Ari has two components, an unchanging black-body-like component which peaks near 3,000 Å, and a rather flat (in frequency units) component which causes the variability. This flat spectrum must also turn down in the optical region. The change between the two spectra is approximately the same (~ 20 mJy) at each wavelength, suggesting the source of the flat spectrum is seen at differing aspects.

It is possible allowing for errors that the whole spectrum from 5,500 to 1,265 has a single amplitude of ~ 1.4 mag. However, the shape of the spectrum lends credence to a change of amplitude with wavelength. It is just conceivable that a disk atmosphere model might explain the whole observed spectral shape from 5,500 to 1,265 Å; this would then all have to vary with the single amplitude. However, the corre-

lation of the UV and X-ray variation makes such a model implausible.

These observations cannot be explained by a hot spot on the edge of an accretion disk. Large variations (amplitude 1.53) are seen at $\lambda = 1,265 \text{ \AA}$; radiation at this wavelength would be expected to arise close in to the white dwarf and not from the edge of an accretion disk. Any modulation by the hot spot is the more difficult when it is remembered that since there are no eclipses the inclination (i) must be $< 60^\circ$. Finally X-ray observations by Jensen *et al.*⁹ rule out a hot spot. They find that 0.1–4 keV X rays vary over the orbital period with an amplitude of ~ 1.5 in a manner similar to the optical variation which they measured simultaneously. They also find X-ray flickering which correlates with the optical flickering, the latter preceding the X-ray flickering by $\geq 40 \text{ s}$. This strange result may be due to a quasi-periodic flickering time of $\sim 40 \text{ s}$ found by Mardirossian *et al.*³. Clearly, the X-ray variation also rules out a model in which the variations arise from a non-axisymmetric atmosphere to the accretion disk, even if a way could be found of maintaining this asymmetry for many orbital cycles so as to produce the light curves. That optical, UV and X-ray radiations all vary in broadly the same manner cannot be consistent with a hot spot model.

This behaviour is exhibited by the AM Herculis stars, and an AM Her model might therefore seem attractive. However, it too can be ruled out. The constant black-body component of the spectrum of TT Ari is distorted by the Balmer jump in emission. Estimating its temperature, therefore, as $\sim 12,000 \text{ K}$ from *UBV* colours rather than from its apparent peak emission at $3,000 \text{ \AA}$ we can then calculate its area (taking $i = 45^\circ$) as $\sim 10^{20} \text{ cm}^2$. There is no room for such a large constant black body in the usual model for an AM Her type system.

The observations do, however, fit a DQ Herculis type model with a slowly (synchronously, or almost so) rotating white dwarf. The constant 'black body' is due to an accretion ring rather than a disk. At the inner edge of this ring the accretion is funnelled down the magnetic field of the white dwarf. The accretion spot at the base of the magnetic accretion columns is thought to have two components (see ref. 10): a hot, optically thin region which produces the hard X rays, and below this a photospheric region radiating most of the accretion luminosity with a black-body-like spectrum ($T \sim 3 \times 10^5 \text{ K}$) which dominates the EUV and the soft X-ray region. If the magnetic field of the white dwarf is offset from the rotation axis the changing aspect of the accretion spot provides a natural explanation for the optical and UV variation. We also note that the absorption lines such as N V, Si V and He II may be formed in the hot

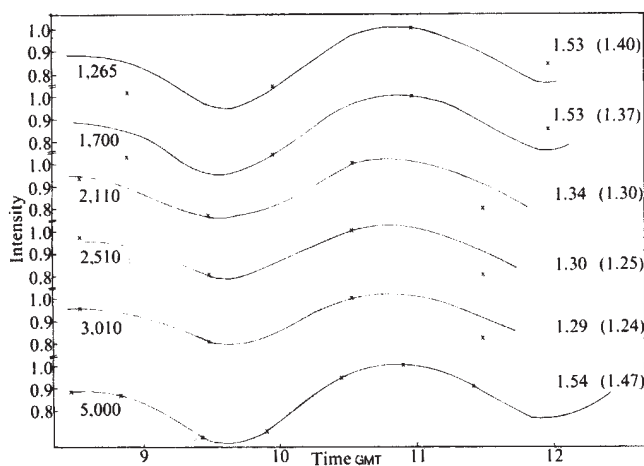


Fig. 2 The 1,265, 1,700, 2,110, 2,510 and 3,010 and 5,000 $\text{\AA}$ light curves of TT Ari. Fluxes are normalized such that maximum light is unity. The numbers on the right give the amplitudes (maximum/minimum) of the curves and in parentheses the amplitudes derived from the observations alone.

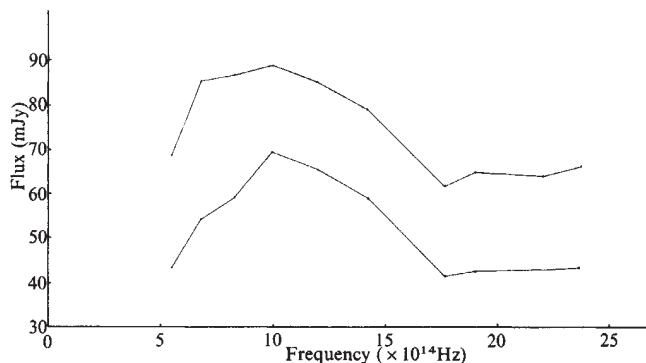


Fig. 3 The maximum and minimum light spectra of TT Ari. 1,360 $\text{\AA}$ and 1,580 $\text{\AA}$ have been added.

photospheric region. More plausibly, any wind flowing from the system may cause these absorption lines.

The sinusoidal modulation of the hard X rays is more difficult to understand as they must come from an optically thin region and there is no evidence for eclipses (we always see just one magnetic pole) or absorption events (K. O. Mason, personal communication). Such sinusoidal modulations are seen in the hard X-ray light curves of some AM Her systems (such as EF Eri = 2A0311–227 (ref. 11), where there is also an absorption event, presumably due to the accretion stream crossing the line-of-sight). It is hard to believe that these modulations are caused by electron scattering by material outside the X-ray source: apart from the difficulty of holding the scattering material in place so as to produce regular light variations, any plausible arrangement will also give photoelectric absorption events, contrary to observation. This cannot be overcome by photoionization, since to remove the K -shell electrons from the absorbing elements requires $L_x \geq 10^3 N_e r^2$, where L_x is the X-ray luminosity, r the distance from the X-ray source and N_e the average electron density in the scattering material. As the scattering optical depth $\tau_{es} \leq N_e r \sigma_T$ must be ≥ 1 and $r \geq 10^{10} \text{ cm}$ in any plausible configuration, we require $L_x \geq 10^3 r \sigma_T \geq 10^{37} \text{ erg s}^{-1}$ to get scattering without absorption, considerably in excess of the total luminosity of TT Ari. This would also be a serious problem for a model invoking hard X-ray production in the boundary layer of an accretion disk extending down to the white dwarf surface. A possible explanation for the hard X-ray variation in an accretion spot model comes from the fact that half of the emitted X rays hit the stellar surface: of these some fraction a_x are scattered outwards again. This scattered (albedo) component will be sinusoidally modulated by an aspect effect just like the soft X rays and EUV. Clearly this idea will work best if a purely scattering albedo ($a_x \sim 0.7$) (ref. 13) as opposed to a scattering plus photoelectric absorption albedo ($a_x \sim 0.3$) (ref. 13) is appropriate.

A second difficulty for our model is the shape of the UV continuum, which is much flatter than the Rayleigh–Jeans continuum ($F_\nu \propto \nu^2$) expected from the accretion spot. We note that in AM Her the UV continuum is $F_\nu \sim \nu^{-1}$, although the eclipsed component does have $F_\nu \propto \nu^2$ (ref. 14). X-ray reprocessing is suspected in AM Her, and may be operating here.

Some cyclotron radiation may be expected from material in the accretion columns. However, because the magnetic field must be weaker than in AM Her this is only likely to contribute in the IR. Furthermore, any such radiation will be heavily diluted by the IR tails of the accretion spot, accretion ring and the red star. Nevertheless, it would be interesting to look for such weak polarization in the IR. TT Ari is known to be unpolarized in the optical region^{15,16}.

The variation of the C IV, Mg II emission lines is puzzling as these must, in any model, come from an optically thin region. Radial velocity studies are needed to establish the likely sites of these emission regions. Modulation could arise if they are

near the heated inner face of the secondary or the base of the accretion columns. We note that large emission line variations are seen in the magnetic rotator EF Eri¹⁷.

The period of rotation of the white dwarf must be the photometric period, 0.1329 days as given by Smak and Stepień⁵. The binary period is, of course, given by the spectroscopic period of 0.13755 days: it would seem therefore that TT Ari has only recently broken away from the synchronism of an AM Her type system.

One might expect to see a reflection effect, due to the reprocessed UV and X rays, off the secondary. This will be best seen when the secondary is viewed over the white dwarf and the magnetic pole of the white dwarf points towards the secondary. This situation will recur every 3.93 days. Observations of such an effect would test our model and confirm the spectroscopic and photometric periods. We note, however, that the predicted ~4-day period is very sensitive to errors in the photometric and spectroscopic periods. In particular if these periods are closer than current values suggest the 'beat' period may become unobservably long.

We also note that the quasi-periodic variations on time scales of about 14 min (ref. 4) may be related to the free fall time down the accretion columns. This would imply an inner orbital period of the accretion ring of ~56 min. This would be variable as the Alfvén radius varies with the kinetic energy density of the ring which in turn depends on the mass transfer rate.

Clearly, our model describing TT Ari cannot be regarded as certain. Many of the problems, however, are caused by a lack of detailed theoretical understanding of the processes associated with accreting magnetized white dwarfs, for example the X-ray variation problem in a non-eclipsing system such as EF Eri. Equally clear is that a classical hot spot cannot explain the UV and X-ray variation. The axisymmetry of models involving an accretion disk with X rays generated at the white dwarf boundary layer makes it very difficult to modulate the short wavelength UV and X rays, because absorption events are not seen and scattering can be ruled out.

Although we have described TT Ari as a DQ Her type system it is perhaps truer to say it is intermediate between the DQ Her and AM Her types. Indeed, one can see a sequence of objects DQ Her (71), H22521035 (805), TT Ari (11,474) and AM Her (synchronized) where the numbers in parentheses represent the spin period of the white dwarf in seconds. Of course, if the spectroscopic period is in error and does equal the photometric period then TT Ari would be an unusual type of AM Her system in which the magnetic field was weak enough to allow the formation of an accretion ring.

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Limits on a radio shell around the Crab Nebula

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It has been suggested¹ that the supernova of AD1054, which produced the Crab Nebula, was of Type II and resulted from the explosion of a massive (10–15 $M_{\odot}$) star. In this picture, the well known optical filaments represent material which originated predominantly in the core of the star and has been accelerated outwards by the pulsar activity. This nebula is supposed to be surrounded by a faint, massive (~10 $M_{\odot}$), high velocity ($>5,000 \text{ km s}^{-1}$) shell² or halo, which was the envelope of the presupernova star. Such an outer halo may have been recently detected at optical wavelengths³. We report here a high sensitivity search at radio wavelengths for the hypothetical outer shell. No shell has been detected and the upper limits indicate that any such shell is fainter than expected from the surface brightness—linear diameter (Σ - D) relation for shell type supernova remnants. Alternative explanations for the optical halo³ are mentioned.

Observations were made in 1975 and 1976 with the Westerbork Synthesis Radio Telescope^{4,5} which then consisted of 12 equatorially mounted paraboloidal antennas distributed over 1.5 km along an east-west line. In searching for a hypothetical radio shell around the Crab Nebula, it is desirable to observe at a low frequency, as the spectrum of the shell is expected to be steeper than that of the pulsar driven nebula, which has $\alpha = 0.26$ ($S \propto \nu^{-\alpha}$) (ref. 6). The frequency used, 610 MHz, provided excellent 'dynamic range' and sensitivity to low brightness features, as well as adequate resolution ($55 \times 151 \text{ arc s}$, $\alpha \times \delta$, FWHM). As the observations were primarily intended for mapping the weak linear and circular polarization of the nebula, a special observing technique was used⁷. The procedure involved two 12-h observations of the Crab Nebula, one with the two perpendicular linearly polarized dipoles in each telescope at position angles (PA) 0° and 90° and the second with them at PA 45° and 135°. Because of the great brightness of the central nebula, it was necessary to attenuate the signals in the parallel channels on the shorter baselines, to prevent saturation in the correlators. Unnecessary loss of signal-to-noise was, however, avoided by performing greater attenuation on the shorter baselines, in effect 'matching' the sensitivity of the instrument to the visibility curve of the radio source. Each of these 12-h observations was sandwiched between two observations, with the same dipole positions, of the calibration source 3C147 to establish accurately the instrumental polarization and by two observations of the same calibrator with the dipoles in their normal positions⁸, to derive the instrumental amplitude and phase corrections. This entire sequence was performed once in March 1975 and again in January 1976, resulting in four independent full (12 h) syntheses of the total intensity distribution. These four maps are in excellent agreement and we use mainly the results of the first series of observations, as the 'dynamic range' is better. Results of the polarization mapping will be presented elsewhere.

At the present resolution the details seen in the highest resolution centimetre wavelength maps^{9,10} are smeared out and the cleaned¹¹ map of the nebula (Fig. 1) shows a smooth, elongated brightness distribution. Averaging the four individual observations, the total flux density is $S_{610\text{MHz}} = 1,085 \pm 30 \text{ Jy}$,

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1. Krautter, J. *et al. Astr. Astrophys.* **98**, 27–29 (1981).
2. Smak, J. & Stepień, K. *Non-Periodic Phenomena in Variable Stars* (ed. Detre, L.) 355–359 (Academic, Budapest, 1969).
3. Mardirossian, F. *et al. Astr. Astrophys.* **85**, 29–35 (1980).
4. Sztano, M. *Inf. Bull. Variable Stars No.* 1710 (1979).
5. Smak, J. & Stepień, K. *Acta astr.* **25**, 379–381 (1975).
6. Cowley, A. P., Crampton, D., Hutchings, J. B. & Marlborough, J. M. *Astrophys. J.* **195**, 413–421 (1975).
7. Jameson, R. F., Sherrington, M. R. & King, A. R. *Mon. Not. R. astr. Soc.* 455–462 (1982).
8. Bailey, J. A. *Mon. Not. R. astr. Soc.* **197**, 31–39 (1981).
9. Jensen, K. *et al. Bull. Am. astr. Soc.* **13**, 818 (1981).
10. Frank, J., King, A. R. & Lasota, J. P. *Mon. Not. R. astr. Soc.* (in the press).
11. Patterson, J., Williams, G. & Hiltner, W. A. *Astrophys. J.* **245**, 618–623 (1981).
12. Hatchett, S., Buff, J. & McCray, R. *Astrophys. J.* **206**, 847–860 (1976).
13. Felsteiner, J. & Opher, R. *Astr. Astrophys.* **46**, 189–195 (1976).
14. Raymond, J. C. *et al. Astrophys. J. Lett.* **230**, L95–L98 (1979).
15. Stockman, H. S., Liebert, J. & Moore, R. L. *Pap. Santa Cruz Summer Workshop on Cataclysmic Variables* (1981).
16. Tapia, S., Priedhorsky, W. & Kzreminski, W. *Pap. at the Summer Workshop on Cataclysmic Variables* (1981).
17. Bailey, J. & Ward, M. *Mon. Not. R. astr. Soc.* **196**, 425–434 (1981).

in good agreement with earlier measurements⁶. The observed major $\times$ minor axes of the nebula (with no correction for beam smoothing) are 4.4×3.1 arc min in $PA = 152^\circ$ at 50% of the peak intensity (FWHM) and 10.4×7.6 arc min in $PA \approx 150^\circ$ at 0.5% of the peak intensity. Assuming a gaussian brightness distribution, the approximate deconvolved¹² sizes are 3.8×2.6 arc min in $PA 134^\circ$ to FWHM, and 8.9×5.6 arc min $PA 124^\circ$ at 0.5%. This deconvolved FWHM is similar to other measurements at centimetre wavelengths⁹. Although the deconvolution is obviously not precise, the size at the 0.5% level may be significantly larger than the total size of 7×5 arc min found at 5 GHz (ref. 9), presumably because of the higher 'dynamic range' of the present data. It is also slightly larger than the optical dimensions of 7×4.8 arc min given by van den Bergh¹³. About 5 arc min to the north of the pulsar, a radio protrusion is seen in the second and third contours. This protrusion lies close to, and slightly to the north of, the optical 'jet-like' feature^{13,14}. However, the low resolution in the declination direction renders the position of the radio feature uncertain and higher resolution data are needed to check for a possible association. The extensions to the north seen in the first contour are probably not real.

To search for a faint radio shell or halo, the sidelobes of the nebula itself were removed from a region 1.5° in radius, centred on the pulsar, by means of the CLEAN algorithm. No significant radio emission of any kind was found within this region on any of the four maps. Because of the residual sidelobe disturbances, we give separate upper limits on map surface brightness, B_M , for three different regions: (1) within a radius $R \approx 7$ arc min centred on the pulsar (but outside the nebular dimensions given earlier) $B_M < 1.0$ Jy (beam area)⁻¹; (2) in two sectors with 7 arc min $< R < 1.5^\circ$, $-10^\circ < PA < 20^\circ$ and $170^\circ < PA < 200^\circ$, the upper limit ranges between $B_M < 0.4$ and $B_M < 1.0$ Jy (beam area)⁻¹; and (3) for the rest of the field (7 arc min $< R < 1.5^\circ$ and outside the above ranges of PA), $B_M < 0.4$ Jy (beam area)⁻¹ everywhere and $B_M < 0.2$ Jy (beam area)⁻¹ in some regions. These last two limits are $\leq 0.2\%$ and $\leq 0.1\%$ of the peak brightness of the Crab Nebula itself. To convert these map limits into surface brightness limits on the sky, B , account must be taken of the decrease in sensitivity of the instrument away from the field centre due to the directional pattern of the individual paraboloids. This relation is

$$B = B_M / \{1 + 0.166R - 2.197R^2 + 1.365R^3 + 0.084R^4 - 0.255R^5 + 0.054R^6\} \quad (1)$$

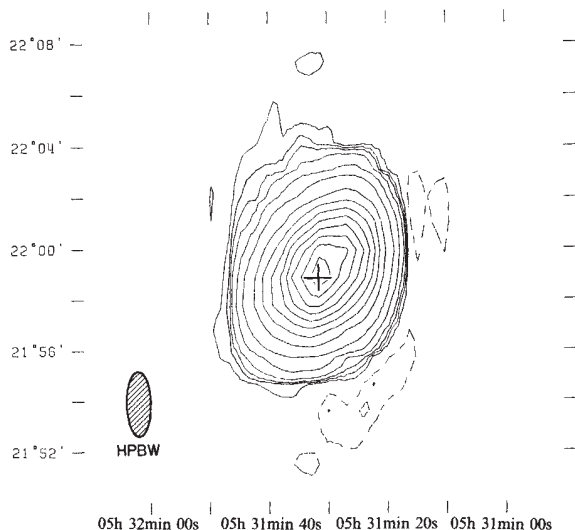


Fig. 1 Contour map of the total intensity distribution in the Crab Nebula at 610 MHz. The beam (55×151 arc s, $\alpha \times \delta$) is shown at the bottom left and the cross marks the pulsar. Contours are plotted at: -2, -1, 1, 2, 3, 5, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180 Jy (beam area)⁻¹. Negative contours are dotted lines, positive contours are solid lines.

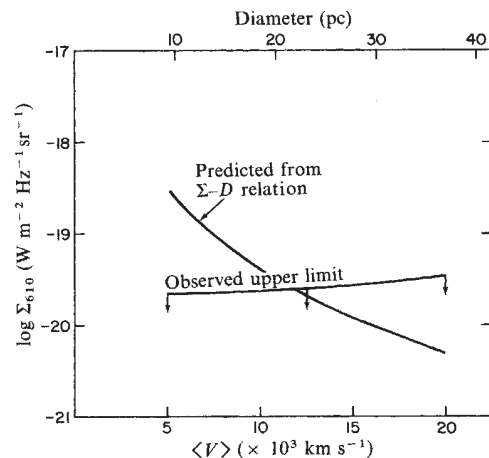


Fig. 2 Plot of surface brightness at 610 MHz (Σ_{610}) against present linear diameter or mean expansion velocity for a hypothetical radio shell around the Crab Nebula at a distance of 2 kpc. The expected mean surface brightness from the Σ -D relation¹⁵ ($|z| = 200$ pc) and the observed upper limit are shown.

where R is the distance from the pulsar ($\alpha(1950.0) = 05$ h 31 min 31.46 s, $\delta(1950.0) = +21^\circ 58' 54.8''$) in degrees. Thus the upper limit on B is just twice the upper limit on B_M at 41 arc min from the field centre and is negligibly (7%) larger than B_M at 14 arc min (the maximum radius of the optical halo³). These upper limits on B may, in turn, be converted into limits on conventional surface brightness by means of

$$\Sigma_{610} (\text{W m}^{-2} \text{Hz}^{-1} \text{sr}^{-1}) = 5.5 \times 10^{-20} B (\text{Jy (beam area)}^{-1}) \quad (2)$$

Thus for the typical upper limit of $B < 0.4$ Jy (beam area)⁻¹, equation (2) implies $\Sigma_{610} \leq 2 \times 10^{-20} \text{W m}^{-2} \text{Hz}^{-1} \text{sr}^{-1}$.

We may compare our limits with the surface brightness-linear diameter (Σ - D) relation for shell-type supernova remnants. While this relationship is empirical and of weak predictive power, it remains the major yardstick for studying the radio evolution of supernova remnants. Using the form determined by Caswell and Lerche¹⁵ and converting from a surface brightness at 408 MHz to one at 610 MHz, assuming a mean spectral index $\alpha = 0.45$ (ref. 16), gives the relation

$$\Sigma_{610} (\text{W m}^{-2} \text{Hz}^{-1} \text{sr}^{-1}) = 0.8 \times 10^{-15} D_{\text{pc}}^{-3} \exp(-|z_{\text{pc}}|/175) \quad (3)$$

where Σ_{610} is the mean surface brightness of the remnant and D_{pc} and z_{pc} are its linear diameter and height above the galactic plane. A shell of radius $14'$ around the Crab Nebula at a distance of 2 kpc ($D = 16.3$ pc) and a height above the galactic plane of $|z| = 200$ pc (ref. 17) would, therefore, be expected to have a mean surface brightness of $\sim 6 \times 10^{-20} \text{W m}^{-2} \text{Hz}^{-1} \text{sr}^{-1}$ and at least a factor of two higher rim brightness for a typical shell. As this value exceeds our radio brightness limit by $>3\sigma$, it is clear that if the optical halo detected by Murdin and Clark³ represents the remnant of the supernova of AD1054, it is atypically weak in the radio range.

A more general way of considering this problem is shown in Fig. 2. The observed upper limit to the 610 MHz surface brightness over most of the map area is shown and compared with the empirical mean surface brightness for shell type remnants from equation (3). As any associated shell is presumed to have an age identical to that of the Crab Nebula itself, an average expansion velocity for each shell size can also be calculated for the abscissa in Fig. 2.

As may be seen, the observed limit lies below the mean expected value for all expansion velocities below $\sim 12,000 \text{ km s}^{-1}$. In fact, because most remnants show appreciable limb brightening, the actual surface brightness in the shell itself would be larger than the mean value computed from the Σ - D relation. Thus the radio observations conflict with the predictions for essentially all plausible expansion velocities.

Therefore, we conclude that any hypothetical shell radio source is fainter than expected from the Σ - D relation. We emphasize that a shell like that of SNR1006 (G327.6+14.5) (ref. 18) would have been barely detected by our observations. However, this source is very faint for its diameter, a fact which may be ascribed to its large distance from the galactic plane ($|z| = 340$, 575 or 1,012 pc, depending on whether the distance is derived from ancient historical records¹⁹, from the $\Sigma(D, z)$ relation¹⁵ or from a $\Sigma(D)$ relation¹⁶), as its properties are not inconsistent with equation (3).

Theoretical treatments of the radio emission from young supernova remnants commonly rely on an interaction between the ejected material and the surrounding interstellar gas to generate the requisite cosmic rays and magnetic fields^{20,21}. In Gull's model²⁰, the radio emission rises to a maximum when the ratio of mass swept up ($= 1.3 < V_{5,000}^3 > n_{0.1} M_{\odot}$ for the Crab, where $n_{0.1}$ is the ambient interstellar density in units of 0.1 cm^{-3}) to mass ejected ($\approx 10 M_{\odot}$ in Chevalier's model¹) is about 0.1. This model suggests, therefore, there should be significant radio emission from the shell at present. Obviously more quantitative models of the evolution of the radio emission from young shell remnants are needed before comparison with our limits becomes meaningful.

Various possible interpretations of the optical halo³ include: (1) emission from the hypothetical remnant shell; (2) a 'Strömgren sphere' in interstellar gas or in gas lost by the presupernova star, which is ionized by the non-thermal UV synchrotron radiation of the nebula²²; (3) light scattered off dust grains surrounding the nebula³; and (4) leakage of synchrotron emitting electrons from the nebula²³. The non-spherical shape of the halo³ may render (2) implausible while any interpretation, such as (3), must be consistent with the apparent absence of excess dust absorption in and surrounding the Crab²⁴, as well as the halo's emissivity in the band straddling $H\alpha$ used by Murdin and Clark³. The most direct way to prove possibility (1) would be the detection of high velocity gas in the halo through optical spectroscopy; in fact, Henry *et al.*²⁵ have recently claimed the detection of gas expanding at velocities of 2,300–7,200 km s^{-1} . Further X-ray observations are also desirable to confirm and map a possible soft X-ray 'halo' peripheral to the nebula²⁶, although this halo is probably the result of scattering of X rays from the central synchrotron source by interstellar dust rather than emission from a hot thermal plasma²⁷.

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Note added in proof: R. B. C. Henry has informed us that the high-velocity emission lines suspected in ref. 25 are of instrumental origin.

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1. Chevalier, R. A. in *Supernovae* (ed. Schramm, D. N.) 53 (Reidel, Dordrecht, 1977).
2. Hazard, C. & Sutton, J. *Astrophys. J. Lett.* **7**, 179 (1971).
3. Murdin, P. & Clark, D. H. *Nature* **294**, 543 (1981).
4. Baars, J. W. M. & Hooghoudt, B. G. *Astr. Astrophys.* **31**, 323 (1974).
5. Casse, J. L. & Muller, C. A. *Astr. Astrophys.* **31**, 333 (1974).
6. Baldwin, J. A. *IAU Symp.* **46**, 22, (1971).
7. Weiler, K. W. & Wilson, A. S. *Astr. Astrophys.* **58**, 17 (1977).
8. Weiler, K. W. *Astr. Astrophys.* **26**, 403 (1973).
9. Wilson, A. S. *Mon. Not. R. astr. Soc.* **157**, 229 (1972).
10. Swinbank, E. & Pooley, G. G. *Mon. Not. R. astr. Soc.* **186**, 775 (1979).
11. Högbom, J. A. *Astr. Astrophys. Suppl.* **15**, 417 (1974).
12. Wild, J. P. *Aust. J. Phys.* **23**, 113 (1970).
13. van den Bergh, S. *Astrophys. J.* **160**, L27 (1970).
14. Gull, T. R. & Fesen, R. A. Preprint (Goddard Space Flight Center, 1982).
15. Caswell, J. L. & Lerche, I. *Mon. Not. R. astr. Soc.* **187**, 201 (1979).
16. Clark, D. H. & Caswell, J. *Mon. Not. R. astr. Soc.* **174**, 267 (1976).
17. Weiler, K. W. & Panagia, N. *Astr. Astrophys.* **90**, 269 (1980).
18. Milne, D. K. *Aust. J. Phys.* **24**, 757 (1971).
19. Minkowski, R. *Astr. J.* **71**, 371 (1966).
20. Gull, S. F. *Mon. Not. R. astr. Soc.* **161**, 47 (1973).
21. Scott, J. S. & Chevalier, R. A. *Astrophys. J. Lett.* **197**, L5 (1975).
22. Apparaio, K. M. V. *Observatory* **93**, 201 (1973).
23. Scargle, J. D. *Publ. astr. Soc. Pacif.* **82**, 388 (1970).
24. Trimble, V. L. *Astrophys. J. Lett.* **18**, 145 (1977).
25. Henry, R. B. C., MacAlpine, G. M. & Kirschner, R. P. Preprint (Univ. Michigan, 1982).
26. Toor, A., Palmieri, T. M. & Seward, F. D. *Astrophys. J.* **207**, 96 (1976).
27. Schattenburg, M. *et al. Astrophys. J. Lett.* **241**, L151 (1980).

Simultaneous radio scattering and white light observations of a coronal transient

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Comprehensive observations of coronal transients have been made using the OSO 7 (ref. 1), Skylab²⁻⁴, Solar Maximum Mission⁵ and currently Solwind^{6,7} coronagraphs. An important question in regard to understanding these transients and their relationship to solar radio burst observations⁸ and *in situ* solar wind measurements⁹ is whether the shock front produced by the disturbances corresponds to the white light front. It has recently been demonstrated¹⁰ that spectral broadening measurements of monochromatic spacecraft radio signals represent a useful tool for detecting and studying shocks near the Sun. We now report that a coronal transient observed by the Solwind coronagraph off the west limb of the Sun on 24 October 1979 was also seen in the spectral broadening observations of the Helios-2 2.3-GHz radio signal. A comparison of these simultaneous data suggests that in this case the shock front does not coincide with the white-light front but in fact precedes it.

Figure 1 shows four coronal images obtained with the Solwind instrument⁶ on 24 October, 1979. The upper images were taken at 0316 and 1258 UT while the lower ones are difference images formed by subtracting the images at 0536 and 0804 UT from that at 0316 UT to show the changes that had taken place in the coronal intensity. The west limb brightening is always present during 0316–0804 UT, but a small loop transient does occur during 0536–0804 UT. The Helios-2 raypath, which is in the ecliptic plane off the west limb of the Sun, moves towards the Sun at the rate of 25 km s^{-1} . 'X' in the 0536–0316 UT difference image marks the location of the Helios-2 raypath at 0536 UT. As can be seen, the Helios-2 raypath is well positioned to observe the transient whose front progressed in an almost westward direction.

Although the front of the moving coronal material is not precisely defined, its speed can be estimated and appears to be very slow. The heliocentric distances of the coronal front at 0536 and 0804 UT are $4.5 \pm 0.5 R_{\odot}$ and $6.0 \pm 1.0 R_{\odot}$ respectively, yielding an outward speed (in the plane of the sky) of $\sim 120 \text{ km s}^{-1}$. However, because of the uncertainties in location, the range of speeds is 0–240 km s^{-1} . Height-time profiles for both the Helios-2 raypath and the white light coronal front measurements are summarized in Fig. 2.

Figure 3 shows the time history of the spectral broadening bandwidth BW of the Helios-2 2.3-GHz signal on 24 October 1979 during 0200–1400 UT. Values of BW, the full width between frequencies enclosing half the area underneath the RF spectrum^{10,11}, were computed using an integration time of 2 min. The increase in BW depicted in Fig. 3 exceeds that which would be expected due to the systematic increase in background corona towards the Sun¹¹. The BW profile in Fig. 3 is similar in shape to that for a shock wave observed by Voyager 1 on 18 August 1979 (Fig. 2a in ref. 10), and although we cannot be certain, the similarity in shape indicates that the event in Fig. 3 is also probably a shock wave. Of course, it is clear from the differences in BW variation and time scale that the Helios-observed shock is substantially less intense and slower than the

Voyager-observed shock. The pre-shock bandwidth of 5 Hz at 2.3 GHz is in agreement with the measurements of the background solar corona near $5R_{\odot}$ (ref. 11).

Bandwidth increases are caused by enhancements in turbulence (electron density fluctuations) or acceleration of the solar wind¹¹ and can be separated by analysing the amplitude scintillations if they are weak¹⁰. Unfortunately, the Helios measurements took place near $5R_{\odot}$, where the 2.3-GHz amplitude scintillations are saturated and strong¹² so that detailed profiles of the wind speed and turbulence cannot readily be obtained as was done in the case of the Voyager measurements¹⁰. The first region of enhanced bandwidth in Fig. 3 (0500–0700 UT) corresponds to the shock front and the compressed turbulent region behind it, while the second broad but lower enhanced region (0700–1400 UT) following the first is probably the turbulent wake.

Based on the coronal transient speed of 120 km s^{-1} determined from the Solwind pictures, the white light coronal front would have crossed the Helios-2 raypath at $5.2R_{\odot}$ around 0700

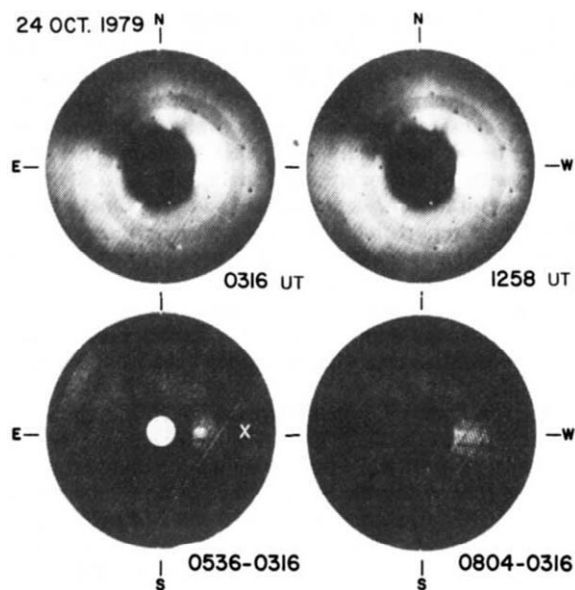


Fig. 1 Coronal images obtained by Solwind on 24 October 1979. The upper images correspond to 0316 and 1258 UT. The lower images are difference images formed by subtracting the image at 0316 UT from the images at 0536 and 0804 UT. 'X' in the 0536–0316 UT difference image marks the location of the Helios-2 raypath at 0536 UT. Note from the spectral broadening data in Fig. 3 that the shock had already crossed the Helios-2 raypath 36 min before 0536 UT.

UT, which is 2 h after the shock arrival time of 0500 UT (see Fig. 3). Alternatively, the 0536–0316 UT difference image in Fig. 1 shows that the white light coronal transient had not reached the Helios raypath at 0536 UT whereas the shock had already been detected 36 min earlier in the Helios radio tracking data. The cross-hatched region in Fig. 2 corresponds to the times at which the white-light front would have crossed the Helios raypath when the range of heliocentric distances at 0536 and 0804 UT is considered. As can be seen, the delay in white light coronal front arrival is at least 1 h for all possibilities. This result is consistent with the fact that path-integrated electron density as determined from radio measurements of phase in the case of the 18 August shock peaks well behind the shock front¹⁰.

Let us estimate the shock speed. Assuming that the white light coronal transient has a constant speed of 120 km s^{-1} , the start time at the Sun's surface would have been 2358 UT on 23 October. As discussed earlier, the shock crossed the Helios raypath at about 0500 UT and according to Fig. 2 the heliocentric distance was $5.5R_{\odot}$. If we assume that the shock originated

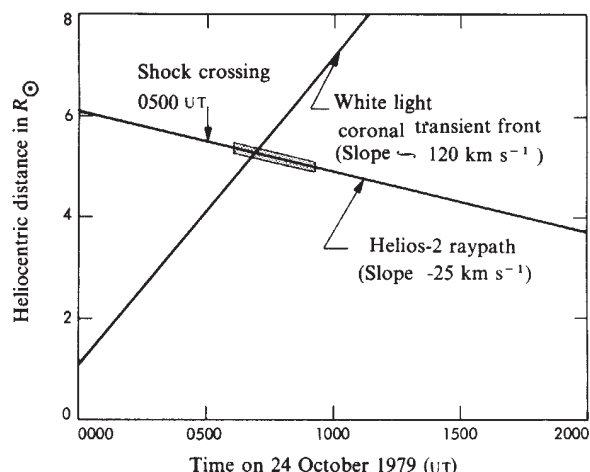


Fig. 2 Height-time profiles for the Helios-2 raypath and Solwind white light coronal front measurements. The cross-hatched region indicates the possible range of intersections between the Helios-2 raypath and the white light transient front allowing for the $120 \pm 120 \text{ km s}^{-1}$ uncertainty in the measured transient speed.

from the Sun's surface at the same time as the white-light front and travelled at a constant speed to $5.5R_{\odot}$, we obtain a shock speed of 170 km s^{-1} . The ambient solar wind speed is about 80 km s^{-1} (ref. 12), so the Helios-observed shock must be rather weak. This result is consistent with the fact that the bandwidth increase is substantially smaller for the Helios than for the Voyager-observed shock. It is interesting that the shock speed calculated on the basis of transit time for the Voyager shock was $2,300 \text{ km s}^{-1}$ (ref. 13), which is a factor of 13.5 higher than that of the Helios shock. This is approximately the same factor relating the time scales of the bandwidth time histories of the Voyager and Helios shocks. The consistency of the Helios and Voyager spectral broadening observations in this regard reinforces the notion that the event observed by Helios is indeed a shock.

Because of the paucity of radio scattering measurements using spacecraft radio signals, there have been few opportunities to compare simultaneous radio scattering and white light measurements of coronal transients. The results presented here are based on only one relatively weak coronal transient but clearly show that the shock front is ahead of the white light front. Additional observations of larger events, particularly when solar radio noise burst and *in situ* spacecraft observations are conducted at the same time, will contribute significantly to our understanding of coronal transients and their evolution as they propagate away from the Sun.

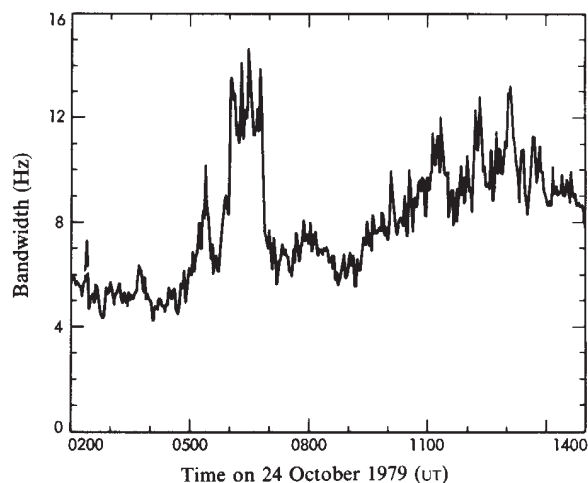


Fig. 3 Time history of Helios-2 2.3-GHz spectral broadening bandwidth BW.

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- Howard, R. A. *et al.* Report UAG, World Data Center A for Solar-Terrestrial Physics (NOAA, Boulder, 1975).
- Gosling, J. T. *et al.* *Solar Phys.* **48**, 389–397 (1976).
- Hildner, E. in *Study of Traveling Interplanetary Phenomena 1977* (eds Shea, M. A., Smart, D. F. & Wu, S. T.) 3–21 (Reidel, Dordrecht, 1977).
- Munro, R. H. *et al.* *Solar Phys.* **61**, 201–215 (1979).
- House, L., Wagner, W. J., Hildner, E., Sawyer, C. & Schmidt, H. U. *Astrophys. J. Lett.* **244**, L117 (1981).
- Sheeley, N. R. Jr, Howard, R. A., Koomen, M. J., Michels, D. J. & Poland, A. I. *Astrophys. J. Lett.* **237**, 199–1101 (1980).
- Poland, A. I., Howard, R. A., Koomen, M. J., Michels, D. J. & Sheeley, N. R. Jr *Solar Phys.* **69**, 169–175 (1981).
- Maxwell, A. & Dryer, M. *Solar Phys.* **73**, 313–329 (1981).
- Gosling, J. T. *et al.* *Solar Phys.* **40**, 439–448 (1975).
- Woo, R. & Armstrong, J. W. *Nature* **292**, 608–610 (1981).
- Woo, R. & Armstrong, J. W. *J. geophys. Res.* **84**, 7288–7296 (1979).
- Armstrong, J. W. & Woo, R. *Astr. Astrophys.* **103**, 415–421 (1981).
- Cane, H. V., Stone, R. G. & Woo, R. *Geophys. Res. Lett.* **9**, 897–900 (1982).

Aquatic chemistry of plutonium in seasonally anoxic lake waters

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We present here data on the distribution of the fallout radionuclides ^{55}Fe , $^{239,240}\text{Pu}$, ^{137}Cs and ^{90}Sr and the natural radionuclides ^{238}U and ^{234}U in a seasonally anoxic lake in an effort to determine their biogeochemical behaviour. Results from Gull Pond, Massachusetts show that the oxygen-depleted and iron- and manganese-rich bottom waters contain five times more dissolved $^{239,240}\text{Pu}$ than oxygenated surface waters. This feature, also observed in anoxic hypolimnia of other lakes, indicates that the concentration of plutonium is significantly influenced by anoxicity and redox cycles. Such observations are important in understanding the fate of artificial radionuclides which are released^{1–4} to the aquatic environment through global fallout, planned disposal and localized accidents.

The oxidation–reduction chemistries of iron and manganese are important in aquatic systems because they can directly or indirectly control the concentration, distribution and speciation of inorganic and organic constituents^{5–7}. In many temperate lakes the development of thermal stratification in summer causes the hypolimnion to become depleted or devoid of dissolved oxygen^{5,8–14}. In such conditions, reductive dissolution of sedimentary Fe(III) and Mn(IV) oxides occurs, releasing dissolved Fe(II) and Mn(II) to the overlying water^{5,8–14}. Anoxic hypolimnia can have large concentrations of dissolved Fe(II) and Mn(II). Ferrous iron is then rapidly oxidized in the oxygenated part of the water column to form suspended particles of Fe(III) oxides^{5–15}.

Many natural radionuclides (such as uranium, thorium, radium, ^{210}Pb , ^{210}Po) and artificial radionuclides (such as plutonium, americium) are known to be strongly adsorbed onto the oxyhydroxides of iron and manganese^{16,17}. Therefore, the

reductive dissolution and oxidative precipitation of iron and manganese oxides in seasonally anoxic lakes may result in changes in the aqueous concentration of several radionuclides. For plutonium and uranium with their multiple oxidation states^{18–25}, variations in redox conditions may lead to changes in speciation and hence concentrations in lake waters.

Gull Pond, a small (0.5 km²) kettle hole lake in Wellfleet, Cape Cod, Massachusetts, develops an anoxic hypolimnion during the late summer (Fig. 1). On 19 August 1981, we collected a vertical profile of water from the deepest basinal part of the lake (22 m depth). Large volume water samples for radiochemical analyses were obtained by pumping water from depth up a plastic hose, through an AMC Cuno Micro-Wynd II filter cartridge with a nominal 1 μm pore size and into 60-l LPE Deldrums. Water samples were filtered without contact with the atmosphere. Such handling is necessary with anoxic waters to prevent the rapid oxidation of dissolved Fe(II) iron to particulate Fe(III) oxide particles. If this reaction did occur before filtration, then the Fe(III) oxides thus formed would probably scavenge dissolved nuclides resulting in the measured dissolved and particulate nuclide concentrations being too low and too high respectively.

Dissolved (<1 μm) radionuclides, iron and manganese were measured on the 2, 15 and 20 m samples. Using Niskin bottles, a series of smaller samples were collected for analysis of dissolved O₂ and total iron and manganese. Oxygen was measured by the Winkler titration and iron and manganese by flame atomic absorption spectrometry. The radiochemical analyses were performed according to published methods— $^{239,240}\text{Pu}$ (ref. 45), U (ref. 46), ^{55}Fe (refs 26, 27), and ^{137}Cs (ref. 28) and ^{90}Sr (ref. 28).

The seasonal anoxicity of the hypolimnion in Gull Pond has been described elsewhere²⁹. During our August sampling (Fig. 1) there was a large decrease with depth in the concentration of dissolved oxygen in the thermocline (9–16 m); and an anoxic hypolimnion existed between 16 m and the bottom (22 m). The oxygen decrease was accompanied by large increases with depth in the concentrations of total iron and manganese. These two elements were predominantly in the dissolved form (<1 μm ; Table 1). The iron and manganese gradients are typical of those observed in other seasonally anoxic lakes^{5,10–12,14}.

The concentration of dissolved $^{239,240}\text{Pu}$ has a 4.6-fold increase between 2 and 20 m with the larger gradient occurring in the anoxic hypolimnion between 15 and 20 m. The gradient for plutonium is much greater than those of dissolved ^{137}Cs , ^{90}Sr and U (Fig. 1). The plutonium profile is more similar to that of ^{55}Fe which has a steep gradient and follows that of stable iron. Iron-55 (half life of 2.7 yr) was introduced with plutonium in the global fallout from atmospheric nuclear explosions testing³⁰. The data from Gull Pond show that dissolved $^{239,240}\text{Pu}$ concentrations are higher in the anoxic hypolimnion. The relative amounts of plutonium in the dissolved and particulate phases have not been measured. Release from the sediments to the water column in anoxic conditions is suggested by the similarities in the plutonium and ^{55}Fe gradients.

The only published studies of plutonium distributions in anoxic lakes are those of Experimental Lake 241, a meromictic Canadian lake with permanently anoxic bottom water^{31–33} and Par Pond (S. C.), a lake with a seasonally anoxic hypolimnion³⁴. In ELA-241 the dissolved $^{239,240}\text{Pu}$ concentration increases from 6 to 10 fCi l⁻¹ between 0 and 9 m, then increases to 48 fCi l⁻¹ at 11 m (Fig. 2). The bottom water has high (but unspecified) concentrations of dissolved iron and manganese and displays a large increase in conductivity^{31–33}. Wahlgren *et al.*^{32,33} have determined that ~80% of the dissolved $^{239,240}\text{Pu}$ in ELA-241 is in the reduced (III + IV) state.

Four vertical profiles (Fig. 2) of $^{239,240}\text{Pu}$ in Par Pond reveal variations which may be linked to redox cycling. The profile in February 1976 shows no vertical gradient when the lake is well mixed. Profiles in July 1975 and August 1976 show the deepest samples up to 20 times more concentrated in plutonium than those in the overlying water. Albert and Orlandini³⁴ reject their

Table 1 Composition of water column profiles in Gull Pond, 19 August 1981

Non-radioactive constituents						
Depth (m)	O ₂ (ml l ⁻¹)	Temperature (°C)	Total Fe (μmol l ⁻¹)	Dissolved* Fe (μmol l ⁻¹)	Total Mn (μmol l ⁻¹)	Dissolved* Mn (μmol l ⁻¹)
0	6.3	23.7	<0.1			
2	—	—	—	<0.2	—	1.1
3	6.6	23.6	<0.1		0.7	
6	6.1	23.4	<0.1		0.4	
9	6.3	23.0	<0.1		0.7	
10.7	7.2	18.8	<0.1		0.4	
12	5.6	14.2	0.3		1.3	
15	—	—	—	54	—	7.3
15.5	0.28	10.5	52		6.9	
19	0	11.0	125		7.3	
20	—	—	—	112	—	6.0
Radioactive constituents—dissolved*						
Depth (m)	^{239,240} Pu (fCi l ⁻¹)	¹³⁷ Cs (fCi l ⁻¹)	⁹⁰ Sr (fCi l ⁻¹)	⁵⁵ Fe‡ (d.p.m. per 100 kg)	²³⁸ U (d.p.m. per 100 kg)	²³⁴ U (d.p.m. per 100 kg)
2	0.17±0.02†	323±2	450±5	0.7±0.8	0.196±0.022	0.205±0.021
15	0.26±0.05	387±2	513±9	29.3±1.1	—	—
20	0.76±0.05	451±4	573±9	63.0±1.2	0.283±0.025	0.329±0.027
Activity ratios of dissolved radionuclides						
Depth (m)	⁵⁵ Fe/ ^{239,240} Pu	⁵⁵ Fe(d.p.m.)/Fe(g)	¹³⁷ Cs/ ⁹⁰ Sr	²³⁴ U/ ²³⁸ U		
2	19±22	—	0.71±0.01	1.05±0.13		
15	514±101	97±4	0.75±0.01	—		
20	371±33	100±2	0.79±0.01	1.16±0.14		

* Dissolved refers to water passing through a Micro-Wynd Cartridge with a 1 μm nominal pore size.

† Precisions are 1σ uncertainties from counting statistics.

‡ ⁵⁵Fe data decay corrected to date of collection (19 August 1981).

high bottom water concentrations of dissolved plutonium in July 1975 as an artefact, do not discuss the high bottom value in total plutonium in the August 1976 profile, and conclude that plutonium is not involved in the redox cycles of iron and manganese.

The results of both studies are ambiguous in that they are not accompanied by basic data indicative of the redox reactions occurring in the lakes at the time of sampling (for example, dissolved O₂, particulate and dissolved iron and manganese). Moreover, it is not clear what precautions were taken to prevent oxidation of dissolved ferrous iron before filtration.

We conclude that the Gull Pond data presented here, supported by the ELA-241 and Par Pond results, indicate that significantly higher dissolved plutonium concentrations are found in anoxic lake waters than in overlying oxic waters. We suggest that the plutonium gradients result from the release of plutonium to water column from the sediments during periods

of anoxia. The exact geochemical processes remain to be established; the possibilities include: (1) plutonium, adsorbed onto sedimentary oxides of iron and/or manganese, is released to the water column as the oxides are reductively solubilized in anoxic conditions^{17,34}; and (2) redox transformations lead to a more soluble oxidation state of plutonium possibly through the formation of organic-Pu(IV) complexes (D. M. Nelson, personal communication); chemical modelling^{24,25} suggests that in anoxic conditions in lakes the concentration of plutonium will increase due to the higher solubility of Pu(III) in equilibrium with Pu₂(CO₃)₃.

The concentrations of ¹³⁷Cs, ⁹⁰Sr, ²³⁴U and ²³⁸U increase only slightly (1.3–1.6-fold) with depth from the surface to the hypolimnetic sample. In comparison with the Great Lakes^{35–38}, the Gull Pond waters have similar concentrations of dissolved ⁹⁰Sr but are greatly elevated in ¹³⁷Cs. The ratios of ¹³⁷Cs/⁹⁰Sr in Gull Pond range from 0.71 to 0.79. This ratio is approxi-

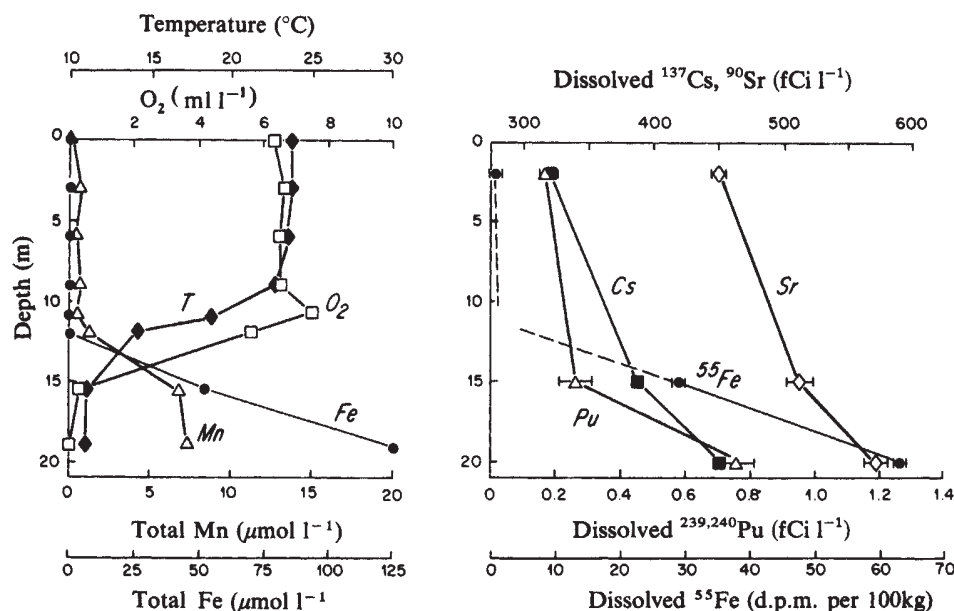


Fig. 1 Chemical composition of the water column in Gull Pond on 19 August 1981. The dashed lines for ⁵⁵Fe, which intersect at 12 m, are based on the more complete profile of stable Fe. Dissolved refers to constituents passing through a Micro-Wynd Cartridge filter with a nominal pore size retention of 1 μm. Water depth at the sampling location was 22 m.

mately half that of global fallout³⁹ (1.6 ± 0.2) and 5–20 times greater than ratios measured in the Great Lakes^{36,37}). The low clay and high sand content of the soils and lake sediments of Cape Cod may lead to diminished exchange capacities and less uptake of dissolved ^{137}Cs from lake and ground waters.

For Gull Pond the water column (0–20 m) inventories of ^{137}Cs and ^{90}Sr are 8 and 10 mCi km^{-2} . These represent about 7 and 12% of the fallout delivery at this latitude. The water column $^{239,240}\text{Pu}$ inventory is $3.5 \times 10^{-3} \text{ mCi km}^{-2}$, only 0.4% of the fallout delivery. These inventory values are calculated for the deepest part of the lake where our samples were taken and thus are upper limits. They indicate that a major fraction of the fallout nuclides are not in the water column.

The different vertical gradients of ^{137}Cs , ^{90}Sr and U relative to Pu and ^{55}Fe imply that there may be different geochemical and/or hydrological processes operating on these groups of radionuclides. It can be argued that the slightly increasing concentrations in Cs, Sr and U may be due to multiple water sources to the pond, redox controlled release from sediments, or biological removal in the surface water and release at depth. In lakes like Gull Pond, which are predominantly ground water fed, the influence of ground water hydrology on the water column chemistry may be an important unknown⁴⁰.

There is some evidence from other anoxic lakes that the Fe/Mn redox cycle may be responsible for increases in the hypolimnetic concentrations of ^{137}Cs (refs 41, 42) and ^{90}Sr (ref. 47). U concentrations are thermodynamically predicted and observed to be very low in anoxic groundwaters^{18,19}. However,

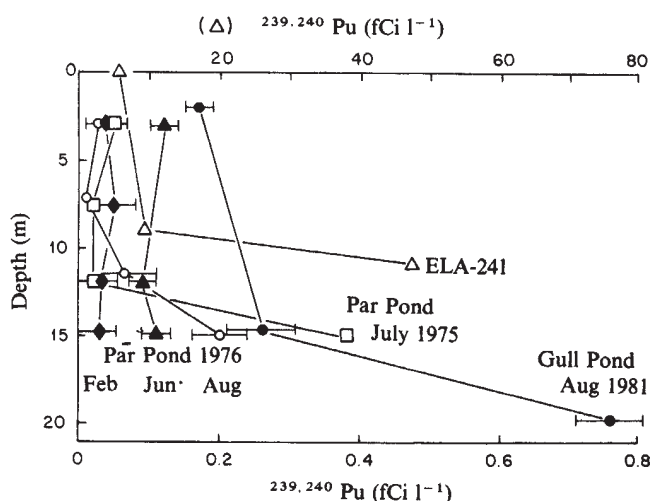


Fig. 2 Profiles of dissolved $^{239,240}\text{Pu}$ in lakes with anoxic hypolimnia. See text for discussion. Water samples from Par Pond (Cold Dam), ELA-241 and Gull Pond were filtered through 0.45, 0.22 and $1.0 \mu\text{m}$, nominal sized filters respectively; the only exception is the August 1976 data from Par Pond which are for unfiltered water samples. The maximum water depths are respectively about 16, 12 and 22 m.

in anoxic marine sediments, U pore water concentrations are elevated over those of oxygenated seawater^{42–44}. The formation of more soluble complexes of U(IV) with organic matter and/or carbonate may be responsible. Because U can exist in several oxidation states and form complexes, it is not unreasonable to expect it to be involved in redox and biogeochemical cycles. This is not usually considered to be the case for Cs and Sr. However, a recent study⁵ of a seasonally anoxic lake shows that cations such as K, Mg, Ca and Ba are indirectly involved in the redox cycles. More geochemical and radiochemical data from seasonally anoxic lakes will elucidate the magnitude of fluxes between sediments and overlying water, the extent of post-depositional remobilization and redistribution, and the interaction between suspended particles and the water.

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1. Harley, J. H. *J. radiat. Res.* **21**, 83–104 (1980).
2. Hardy, E. P., Krey, P. W. & Volchok, H. L. *Nature* **241**, 444–445 (1973).
3. Cleveland, J. M. & Rees, T. F. *Science* **212**, 1505–1509 (1981).
4. Rees, T. F., Cleveland, J. M. & Gottschall, W. C. *Envir. Sci. Technol.* **12**, 1085–1087 (1978).
5. Sholkovitz, E. R. & Copland, D. *Geochim. cosmochim. Acta* **46**, 393–410 (1982).
6. Stumm, W. & Morgan, J. J. *Aquatic Chemistry* (Wiley, New York, 1981).
7. Morgan, J. J. & Stumm, W. *Proc. 2nd Int. Conf. on Water Pollution Research* (Pergamon, Oxford, 1964).
8. Mortimer, C. H. *J. Ecol.* **29**, 280–329 (1941); **30**, 147–201 (1942).
9. Delfino, J. J. & Lee, G. F., *Envir. Sci. Technol.* **2**, 1094–1100 (1968).
10. Davison, W. *Limnol. Oceanogr.* **22**, 746–753 (1977).
11. Davison, W. & Heaney, S. I. *Limnol. Oceanogr.* **23**, 1194–1200 (1978).
12. Davison, W., Heaney, S. I., Talling, J. F. & Rigg, E. *Schweiz. Z. Hydrol.* **42**, 196–224 (1981).
13. Gorham, F. *Limnol. Oceanogr.* **3**, 291–298 (1958).
14. Davison, W. *Nature* **290**, 241–243 (1981).
15. Pankow, J. F. & Morgan, J. J. *Envir. Sci. Technol.* **15**, 1155–1164, 1306–1313 (1981).
16. Bacon, M. P., Brewer, P. B., Spencer, D. W., Murray, J. W. & Goddard, J. *Deep-Sea Res.* **27A**, 119 (1980).
17. Edgington, D. N., Alberts, J. J., Wahlgren, M. A., Karttunen, J. O. & Reeve, C. A. *IAEA Symp. (SM-199/47)* 493–516 (1976).
18. Osmond, J. K. & Cowart, J. B. *Atomic Energy Rev.* **14**, 621–679 (1976).
19. Cowart, J. B. & Osmond, J. K. *Isotope Techniques in Groundwater Hydrology* 131–149 (IAEA, Vienna, 1974).
20. Hussain, N. & Krishnaswami, S. *Geochim. cosmochim. Acta* **44**, 1287–1291 (1980).
21. Cleveland, J. M. *Am. Chem. Soc. Symp. Ser. No. 93*, 321–333 (1979).
22. Aston, S. R. *Mar. Chem.* **8**, 319–325 (1980).
23. Nelson, D. M. & Lovett, M. B. *Nature* **276**, 599–601 (1978).
24. Allard, B. *J. Inorg. Nucl. Act.* **42**, 1015–1027 (1980).
25. Allard, B. *Proc. Actinide 81 Conf.* (ed. Edelstein, N.) (Pergamon, Oxford, in the press).
26. Labeyrie, L. D., Livingston, H. D. & Bowen, V. T. *IAEA Symp. (SM-199/115)*, 121–136 (1976).
27. Labeyrie, L. D., Livingston, H. D. & Gordon, A. G. *Nucl. Instrum. Meth.* **128**, 575–580 (1975).
28. Wong, K. M., Noshkin, V. E. & Bowen, V. T. *IAEA Tech. Rep. Ser. No. 118*, 119–127 (1970).
29. Soukup, M. A. *Univ. Mass.-National Park Serv. coop. Res. Unit Rep.* **35**, (1977).
30. Weimer, W. C. & Langford, J. C. *Atmos. Envir.* **12**, 1201–1205 (1978).
31. Wahlgren, M. A., Alberts, J. J., Orlandini, K. A. & Kucera, E. T. *Argonne natn. Lab. A. Rep. ANL-77-65*, 92–94 (1977).
32. Wahlgren, M. A. *et al. Argonne natn. Lab. A. Rep. ANL-77-65*, 95–98 (1977).
33. Wahlgren, M. A., Nelson, D. M., Orlandini, K. A. & Larson, R. P. *Argonne natn. Lab. A. Rep. ANL-78-65*, 64–68 (1978).
34. Albert, J. J. & Orlandini, K. A. *Geochim. cosmochim. Acta* **45**, 1931–1939 (1981).
35. Wahlgren, M. A. & Marshall, J. S. *IAEA Symp. (IAEA-SM-199/47)*, 227–243 (1975).
36. Wahlgren, M. A. & Nelson, D. M. *Verein. Limnol.* **19**, 317–322 (1975).
37. Alberts, J. J. & Wahlgren, M. A. *Envir. Sci. Technol.* **15**, 94–98 (1981).
38. Alberts, J. J., Wahlgren, M. A., Nelson, D. M. & Jahn, P. J. *Envir. Sci. Technol.* **11**, 673–676 (1977).
39. Hardy, E. & Chu, N. *Atomic Energy Comm. Health and Safety Lab. Rep. HASL-182*, 1–6–1–9 (1967).
40. Frape, S. K. & Patterson, R. J. *Limnol. Oceanogr.* **26**, 500–517 (1981).
41. Alberts, J. J., Till, L. T., & Vigerstad, J. J. *Science* **203**, 649–651 (1979).
42. Baturin, G. N. *Geochim. Int.* 1031–1041 (1973).
43. Boulad, A. P. & Michard, G. *Earth planet. Sci. Lett.* **32**, 77–83 (1976).
44. Kolodny, Y. & Kaplan, I. R. in *Proc. Symp. Hydrogeochemistry and Biogeochemistry* (Vol. 1 (ed. Ingerson, E.)) 418–442 (Clarke Co., 1973).
45. Livingston, H. D., Mann, D. R. & Bowen, V. T. *Am. Chem. Soc. Adv. Chem. Ser.* **147**, 124–128 (1975).
46. Krishnaswami, S. & Sarin, M. M. *Analyt. chim. Acta* **83**, 143–156 (1976).
47. Torgersen, T., Hammond, D. E., Clarke, W. E. & Peng, T. H. *Limnol. Oceanogr.* **26**, 110–122 (1981).

Preferential elution of strong acids from a Norwegian ice cap

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The release of strong acids by melting snows during the early thaw has a serious effect on the ecology of many sensitive areas of the world^{1–3}. The run off is a product of both the input (wet and dry deposition) and of processes occurring within the snow-pack and the firn during the melt season. Temperate glacial ice is very pure⁴, having lost most of its annual input of original solutes during each melt season, and the analyses reported here of the ionic concentrations in the snow, firn and ice of the top 20 m of an ice cap, indicate that the ions are eluted differentially and in a manner which initially accentuates the loss of sulphuric and nitric acid. Almost all the annual deposits of ions on a glacier or ice cap may be lost during a single year.

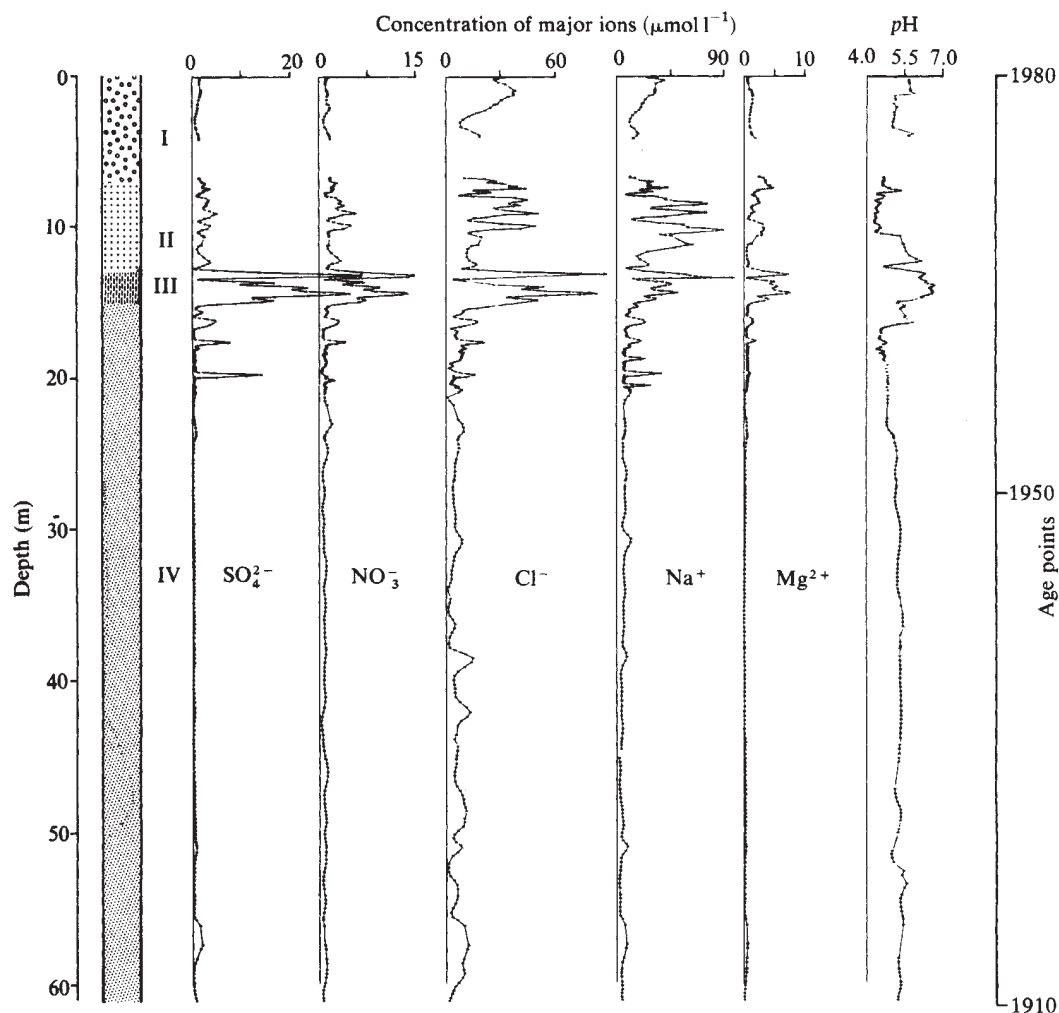


Fig. 1 Distribution of major ions down the ice core; the data have been smoothed using a 5-point binomial filter. Structure of core: I, snow and firn; II, firn and open-structured ice; III, ice with vertically-connected bubble system; IV, bubbly ice. The age points are based on climatically-reconstructed accumulations, simple flow models and tritium determinations.

An ice core, 61 m long, was recovered from the secondary summit (1,625 m ASL) of the Folgefonni Ice Cap (60°04' N, 06°23' E) near Bergen, Norway at the end of July 1980. The annual net accumulation at the drilling site is about 1 m water equivalent. Precipitation would be 3–4 times this amount and have approximate inorganic ion concentrations of: sodium, 100; chloride, 115; nitrate, 22; sulphate, 24; magnesium, 10; hydrogen, 35; ammonium, 15; calcium, 8 $\mu\text{mol l}^{-1}$ (ref. 5). The core was retrieved using a hand auger for the upper snow and firn and a portable thermal corer⁶ for the more consolidated firn and ice. The core was returned to the UK in its frozen state for analysis. About 1 m of core between 6 and 7 m was lost during the transfer from hand auger to thermal corer. The overall structure of the core was: 0–7 m snow and firn, 7–13 m firn and relatively open-structured ice, 13–15 m a zone of ice with systems of vertically interconnected bubbles, below 15 m bubbly ice (Fig. 1). The retrieved core (7-cm diameter) was cut into disks (5-cm thick). Before analysis, an outer 1-cm annulus was removed and the remaining central sections sluiced with deionized water until about half of the original volume remained. The firn sections were cut using carefully cleaned polytetrafluoroethylene (PTFE) tools and not washed with deionized water. All samples were filtered through 0.22- μm nucleopore filters. The anionic components were determined on a Dionex 12 ion chromatograph. Concentrations of the sulphate and nitrate anion were determined to better than 10% accuracy in the 1–10 $\mu\text{mol l}^{-1}$ range with a detection limit of 0.1 $\mu\text{mol l}^{-1}$. Chloride levels were determined to 10% accuracy with a 0.5 $\mu\text{mol l}^{-1}$ detection limit. Sodium and magnesium were determined on a Pye Unicam SP9 atomic absorption spectrometer; accuracy was ~5% and detection limits better than 0.5 $\mu\text{mol l}^{-1}$. The H^+ ion concentration was determined

with a pH meter read to 0.1 pH unit. The ionic composition of the core below 20 m was more constant relative to the upper 20 m and analyses were made only on every fifth disk.

The distribution of major ions down the core is shown in Fig. 1. The few NH_4^+ , Ca^{2+} and K^+ determinations available (the small volume of the ice disks precluded complete ion analysis) indicate that these ions probably provide the necessary charge for balance. The concentration of ions in the ice below 20 m are generally an order of magnitude lower than that which would be expected from the composition of the precipitation input. This is in agreement with earlier observations^{7,8}. More striking is the high peak in concentrations at 12–15 m, where the ice had many vertically interconnected bubbles. The peak is most pronounced for nitrate and sulphate. Below the water table (20 m) the concentration of ions is very low, which suggests that the bulk of the inorganic solutes are removed from the ice cap. Summing the ions present above this point yields approximately: SO_4^{2-} , 0.070; NO_3^- , 0.050; Cl^- , 0.40; Na^+ , 0.46; Mg^{2+} , 0.030 mol m^{-2} . These values are of the same order as a single year's input of inorganic ions for such an area of southwestern Norway, indicating that much of the ion input since the 1979 melt season had not yet (at the time of core retrieval) been removed from the ice cap, although it has been redistributed through deeper (and older) ice. The melt water percolates down through the upper open-structured layers of the ice cap each year, taking the bulk of the ionic components of that year's input with it. These reach the water table and are ultimately lost from the ice cap, eventually leaving residual concentrations of the level in the lowest 40 m of the core.

However, the percolation does not occur without redistribution among the ions. The ratios of the volume-averaged concentrations of ions in the top 3 m of the core, C_{0-3} (representing

the remaining winter input), to the average ionic concentrations in the entire top 20 m of the core, C_{0-20} are: SO_4^{2-} , 0.39; NO_3^- , 0.58; Cl^- , 1.20; Na^+ , 0.90; Mg^{2+} , 1.15. These values may be seen as some kind of retention index (a proportion of the ions, particularly the more mobile ones, may already have left the system and this will distort the calculated values), and suggest that some ions are eluted from the snow and firn more rapidly than others. Differential enrichment of the ions is also apparent in the vertically ducted ice zone (C_{12-15}/C_{0-20}): SO_4^{2-} , 3.8; NO_3^- , 2.5; Cl^- , 1.9; Na^+ , 1.4; Mg^{2+} , 3.5. Our examination of data from snowpack studies⁸⁻¹⁰ show similar rankings in terms of ion retention, with Cl^- and Na^+ being retained longer, NO_3^- retained for shorter periods and SO_4^{2-} and Mg^{2+} being the most mobile of the measured ions. Thus, the fractionation process (removal of the bulk of the impurities from the snowpack in the first fractions of meltwater) that has received much comment⁷⁻¹⁰, is also a differential process, releasing some ions more quickly than others.

The average ion concentrations of various sections of the ice core are shown in Fig. 2. Note that Na^+ and Cl^- seem to be less mobile than the NO_3^- and SO_4^{2-} in the ice column. Our examination of snowpack data⁸⁻¹⁰ also indicates the high mobility of the H^+ ions which probably therefore accompany the mobile NO_3^- and SO_4^{2-} ions as a strong acid peak appearing relatively early in the melt season. A hint of this process may be seen at the 12–15-m peak, where the SO_4^{2-} and NO_3^- concentrations are high, and the removal of each SO_4^{2-} and NO_3^- ion would lead to the associated removal of three H^+ ions. The H^+ concentration is low in this part of the core and the charge is balanced by NH_4^+ and K^+ (which, on the basis of the measurements made so far, appear to be relatively immobile). The C_{0-3}/C_{20} value for H^+ is 0.48, confirming the high mobility of the ion, but the distribution of H^+ down the ice core does

indicate the complexity of the removal process, and the effects of buffering might conceal even larger H^+ ion mobilities. If a melting season was reasonably long then we would expect the more slowly removed Na^+ and Cl^- ions to appear after the acids, making the total removal of ions equal to the input, maintaining a steady-state condition. However, short melting seasons might allow temporary retention of the less mobile constituents. The fractionation has previously been observed in snowpacks, but here we can see it occurring in a vertically extended ice core in a differential manner. It does allow us to perceive the way in which the 'acid flush' may arise from an ice cap, a situation which has some similarities with snow melt and is of obvious importance to the vernal acidification of watercourses.

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1. Hagen, A. & Langeland, A. *Envir. Pollut.* **5**, 45–57 (1973).
2. Leivestad, H., Hendry, G., Muniz, I. P. & Snekvik, Y. in *Impact of Precipitation on Forest and Freshwater Ecosystems in Norway* (ed. Broekke, F. H.) 86–111 Res. Rep. 6/76, (SNSF-Project, Aas, 1976).
3. Leivestad, H. & Muniz, I. P. *Nature* **259**, 391–392 (1976).
4. Glen, J. W., Homer, D. R. & Paren, J. G. in *Isotopes and Impurities in Snow and Ice Symp.*, 263–268 (IAHS-AISH publ. 118, International Association for Hydrological Science, 1977).
5. *Data 1979, 1980 from Norwegian Monitoring Programme for Long Range Transported Pollutants in Air and Precipitation* (Norwegian Institute for Air Research, Lillestrøm).
6. Davies, T. D., Brimblecombe, P. & Vincent, C. E. *Weather* **32**, 386–390 (1977).
7. Overreim, L. N., Seip, H. M. & Tollan, A. in *Acid Precipitation—Effects on Forests and Fish*, 77–90 (Final Rep. SNSF-Project, Aas, 1980).
8. Johannessen, M. & Henriksen, A. *Wat. Resour. Res.* **14**, 615–619 (1978).
9. Skartveit, A. & Gjessing, Y. T. *Nordic Hydrol.* **10**, 141–154 (1979).
10. Johannessen, M., Skartveit, A. & Wright, R. F. in *Ecological Impact of Acid Precipitation* (eds Drablos, D. & Tollan, A.) 224–225 (SNSF-Project, Aas, 1980).

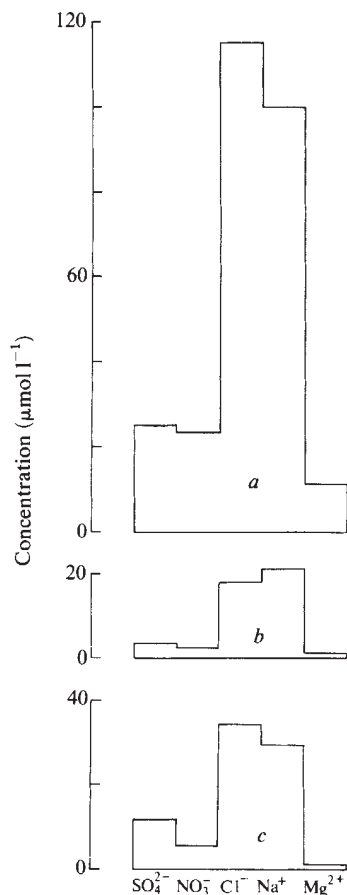


Fig. 2 a, Composition of precipitation input on the glacier since 1979 melt season, estimated from Norwegian Institute for Air Research data. b, Average composition of 0–20 m zone of ice core. c, Average composition of 12–15 m zone of ice core.

Stable isotopic evidence for latest Miocene sea-level fall in the Mediterranean region

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The discovery of a marked isochronous decrease of carbon isotope ratio values (~ 0.5 – 0.8 ‰ in marine carbonates at about 6.2 Myr has stimulated several speculations regarding the origin of this event^{1–3}. Here we report stable isotope studies of planktonic and benthic foraminifera from the late Miocene Carmona-Dos Hermanos section in southwestern Spain which suggest that (1) the carbon shift at 6.2 Myr is directly associated with a eustatic sea-level lowering recorded in the same section; (2) the shift in $\delta^{13}\text{C}$ at 6.2 Myr and other times may be related to an increased flux of marine and terrigenous organic matter depleted in ^{13}C and eroded from the continental shelves during sea-level lowstands; and (3) the sea-level fall at 6.2 Myr triggered the rapid shift towards hypersaline conditions in the Mediterranean well before the deposition of the Messinian evaporites.

Climatic and oceanographic events that may be closely associated in time with the decrease in $\delta^{13}\text{C}$ values include: (1) an overall cooling of surface water^{4,5}; (2) an increase in bottom

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Table 1 Stable isotopic results from the Carmona-Dos Hermanos section, southwestern Spain

Sample no.	Thickness(m)	<i>Orbulina</i> <i>Universona</i>		<i>Globigerinoides</i> <i>obliquus</i>		<i>Cibicides</i> <i>mediocris</i>		<i>Uvigerina</i> sp.		<i>Globicassidulina</i> <i>subglobosa</i>	
		$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	$\delta^{13}\text{C}$
C17	410.0	-0.98	1.03	-1.85	0.82						
		-0.90	0.96	-2.06	0.64						
C12	305.0	-0.83	0.83	-2.10	0.78	-0.10	-0.87	0.48	-1.51		
		-0.80	0.72	-1.93	0.81	-0.12	-0.59				
C11	292.0	0.00	0.93			0.41	-0.35	1.24	-1.24	0.96	-0.81
C10	280.0	-0.93	2.04	-1.14	1.69	0.47	0.27	1.10	-0.83	0.89	-0.53
C9	260.0	-0.53	1.94	-1.28	1.69	0.15	0.40	0.85	-0.58	0.83	-0.05
C8	240.0	-0.73	2.19	-0.40	1.56	0.17	0.02	1.15	-0.62	1.15	0.40
C7	225.0	-0.16	1.67			-0.03	-0.23	0.63	-0.59	0.66	-0.21
C6	203.0	0.02	2.25	-1.06	1.78	0.61	0.13	0.83	-0.54	0.76	-0.11
								0.95	-0.70		
C5	184.0	-0.42	1.33			-0.14	-0.19	1.11	-0.85	0.64	-0.47
C4	165.0	-0.73	1.68			0.15	-0.12				
C3	153.0	-0.70	2.27	-1.63	1.57	-0.21	0.25	0.57	-0.58	0.44	-0.34
C2	130.0	0.08	1.99	-1.67	2.17	0.46	0.35	0.99	-0.49	0.75	-0.14
C1	110.0	0.01	1.99	-0.89	1.49	0.21	0.34	0.88	-0.54	0.63	-0.33
		-0.54	2.54								
AG3	20.0	-0.37	1.80	1.56	1.17	0.29	1.07	0.69	-0.64		
AG2	0.0	-0.57	1.44			0.05	0.65	0.69	-0.70	0.47	0.15

water circulation rates and fertility of the oceans²; (3) lowering of sea level^{6,7}; (4) the isolation and drying up of the Mediterranean^{8,9}; (5) the shoaling of the Isthmus of Panama¹⁰; (6) an increase in biogenic silica removal in the Southern Ocean high productivity zone¹¹; (7) a decrease in biogenic silica sedimentation in the eastern equatorial Pacific¹²; (8) a change over from an Atlantic carbonate compensation depth (CCD) shallower than the Pacific CCD before the shift to a deeper CCD in the Atlantic after the shift¹³; and (9) an increase in deep-sea accumulation rates¹⁴. The cause of the decrease in $\delta^{13}\text{C}$ values and its apparent close temporal association with these other palaeoceanographic phenomena has not been established.

One way to decrease oceanic $\delta^{13}\text{C}$ values is to introduce isotopically light (-20 to -25%) organic matter to the open ocean by eroding it from the continental shelves during a sea-level lowstand^{2,15}. A eustatic lowering of sea level has been reported close to the time of the carbon shift^{6,9,16}. However, because the carbon shift has mostly been reported from deep-sea drilled sections and eustatic sea-level lowerings are documented in shallow land-based marine sections, direct correlation has proved difficult. Our stable isotopic study of marine carbonates from a land-based marine section in southwestern Spain reveals an abrupt $\delta^{13}\text{C}$ shift which coincides with a previously recorded latest Miocene sea-level fall⁶.

The Guadalquivir Basin in southwestern Spain (Fig. 1) appears to represent a fully marine equivalent of the Messinian evaporite sequence⁶. Its position in the Betic Straits region in southern Spain which acted as a gateway controlling water

movements between the Atlantic Ocean and the Mediterranean Sea during the Miocene⁸ and its shallow depth make it ideal for studying the relationship between sea-level events, climatic changes and Messinian events in the western Mediterranean region.

The Carmona-Dos Hermanos section consists of three units (Fig. 2). The lower 'blue marls' or 'marne azzure' are a thick sequence of marls containing abundant planktonic and some benthic foraminifera and are overlain by a band of alternating sands and marls—'sandy marls'. The upper unit known as the Caliza Tosca is a coarse-grained limestone containing abundant molluscan shell fragments and a high percentage of quartz.

Berggren and Haq⁶ examined the distribution of benthic foraminiferal assemblages in the Carmona section and estimated changes in bathymetry based on comparisons with the depth distribution of similar living assemblages. Below the Caliza Tosca the benthic foraminiferal fauna is relatively homogeneous, but between samples C9 and C12 (Fig. 2) there is a sequential elimination of certain species. Between samples C10 and C13 benthic foraminifera species diversity drops from 46 to 3 species. The dramatic benthic faunal changes at this location, between samples C9 and C13, have been interpreted to reflect a local sea-level fall of ~ 200 m due to the combined influence of long term uplift in the Betic region and more rapid global causes⁶.

Carbon isotopic ratios in both planktonic and benthic foraminifera decreased dramatically by nearly 1‰ between samples C10 and C12, reflecting a change in seawater isotopic

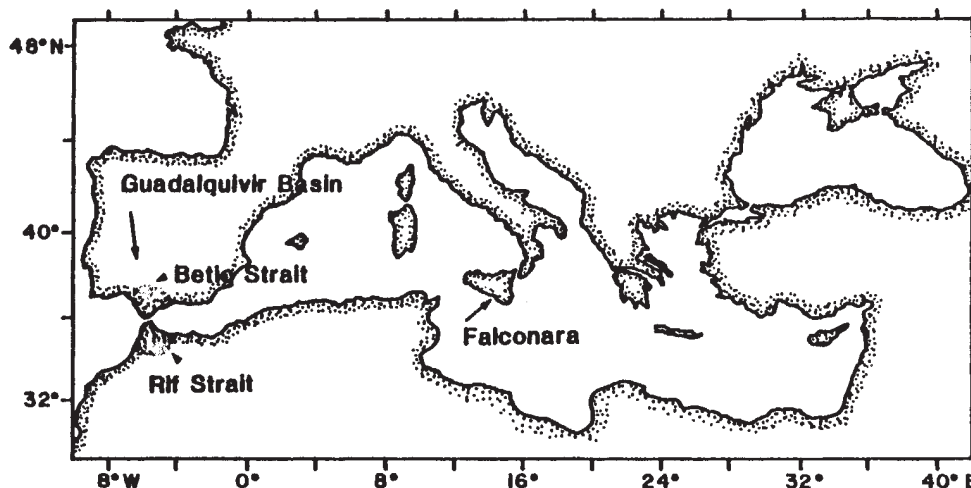
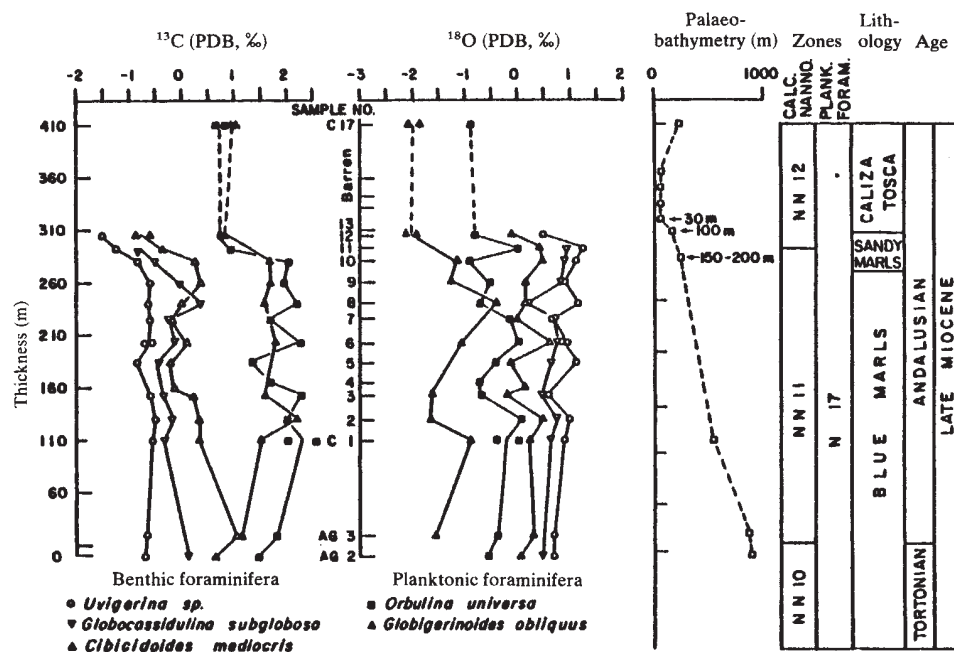


Fig. 1 Map of the Mediterranean Sea and surrounds showing the location of features referred to in the text (modified after ref. 30).

Fig. 2 Benthic and planktonic oxygen and carbon stable isotopic results (‰, PDB), palaeo-depth estimates⁶ and microfossil zonation schemes and lithology⁶ from the late Miocene Carmona-Dos Hermanos section in south-western Spain. Two planktonic foraminiferal species, *Orbulina universa* and *Globigerinoides obliquus* and three benthic foraminiferal species, *Uvigerina* sp., *Globocassidulina subglobosa* and *Cibicidoides mediocris*, were picked from the 250–350 µm size fraction and analysed according to standard procedures¹⁷. Results are presented in the notation

$$\delta\text{‰} = [(R_{\text{sample}}/R_{\text{reference}}) - 1] \times 10^3$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{18}\text{O}/{}^{16}\text{O}$, respectively, for $\delta^{13}\text{C}$ or $\delta^{18}\text{O}$ with respect to PDB (see ref. 17).



composition (Fig. 2, Table 1). An estimate of the timing of the $\delta^{13}\text{C}$ shift in the Carmona section is critical to understanding its origin. Is it related to the latest Miocene carbon isotopic shift reported^{2,3,17} in the Indian, Pacific and Atlantic Oceans? Based on the Berggren and Haq study⁶ the carbon shift recorded in the Carmona section appears to occur within the lower part of the Messinian between 6.5 and 5.5 Myr. It is difficult to define the age of the $\delta^{13}\text{C}$ decrease at this location because the stratigraphical position of each biostratigraphical datum is poorly defined (± 15 m) due to wide sample spacing and the reliability of each datum when applied in the Mediterranean region is questionable. The placement of the Caliza Tosca as being coeval with the Mediterranean evaporites is based on stratigraphical position and the absence of *Discoaster quinqueramus* from one sample (C12) rather than on positive evidence and is therefore also questionable⁹. The widespread Epoch 6 marker *Amaurolithus primus* found below the carbon shift in many Pacific sections is not present in the Carmona section.

The latest Miocene open ocean carbon shift is dated palaeomagnetically at ~ 6.2 Myr within magnetic chron 6^{6,18,20}. On the basis of biostratigraphical criteria, we cannot state that the carbon isotopic shift in the Carmona section is time-equivalent with the open ocean shift. On the other hand, the similarity in age of the isotope shifts, the fact that the shifts occur in both planktonic and benthic foraminifera simultaneously and that the magnitude of the shifts is similar, suggest that a globally synchronous geochemical event which affected both shallow and deep ocean waters is also recorded in the Mediterranean region.

Oxygen isotope results at the Carmona section reveal that the magnitude of the average difference between values from the benthic foraminifera *Uvigerina* and the shallow dwelling planktonic foraminifera *Globigerinoides obliquus* is nearly constant and $>2\text{‰}$ (Table 1, Fig. 2). This isotopic difference probably reflects a surface to bottom temperature contrast of 8–9 °C because the benthic foraminifera fauna (*Bulimina*, *Discorbis*, *Cibicides* and *Melonis*) suggest normal marine rather than hypersaline conditions. Also the presence of psychrospheric ostracods in the middle to late Miocene Mediterranean Sea indicate cool bottom water temperatures²¹. Similar planktonic and benthic foraminiferal $\delta^{18}\text{O}$ differences are found elsewhere in the Mediterranean²².

One possible way of rapidly decreasing organic $\delta^{13}\text{C}$ values in the latest Miocene is by introducing oxidized organic matter or increasing the rate of flux of carbon from a major carbon

reservoir to the ocean^{2,15}. Such changes could be produced by increasing the oxidation of organic matter on the deep ocean sediment surface as a result of increased benthic erosion and higher oxygen content in deep waters^{1,3}. However, Broecker¹⁵ has suggested that the cycle of production, deposition and removal of continental shelf sediments during transgressions and regressions across the shelf could produce a significant change in open ocean $\delta^{13}\text{C}$ values. The shelves act as a sink for carbon during transgressions and a source during regressions, when erosion supplies previously deposited organic matter, depleted in ${}^{13}\text{C}$, to the open ocean^{2,15}.

Close temporal association of the distinct global negative carbon isotopic shift and a eustatic lowering of sea level in the latest Miocene suggest that the two may be causally linked. In addition the correspondence between sea-level changes and a negative $\delta^{13}\text{C}$ shift in marine carbonates recorded at Carmona has been noted in two New Zealand land sections: Blind River and Eketahuna¹⁸. Many late Miocene sections record significant lowerings of sea level evidenced by a global lack of shallow water carbonates, facies changes in ostracod faunas and benthic foraminifera⁹ and marine phosphorite deposits²³. Palaeontological correlation of such events is especially difficult because of the removal of continental margin sequences by erosion, the short-time interval involved and poor micropalaeontological control in many continental margin sequences. The sea-level fall is estimated to have been between 70 and 100 m and to have occurred rapidly ($<10^4$ yr). Such rapid changes in sea level are inferred to have been caused by glacio-eustatic events. Based on oxygen isotopic analyses of marine carbonate in DSDP Site 284, Shackleton and Kennett²⁴ suggested that an increase in Antarctic ice-volume sufficient to produce a sea-level fall between 40 and 70 m occurred in the latest Miocene. However, further isotopic analysis of many late Miocene DSDP drill sites and piston cores have found $\delta^{18}\text{O}$ changes similar in magnitude to that recorded at Site 284 throughout the late Miocene, but no permanent change in oxygen isotopic values^{17,25,26}. This apparent incompatibility between stratigraphical, lithological and oxygen isotopic evidence for a latest Miocene sea-level change hinders a full understanding of late Miocene palaeoceanography. We suggest two possible mechanisms for this incompatibility: (1) the oxygen isotopic record is missing during the critical time due to unconformities and (2) large amounts of continental ice, more enriched in ${}^{18}\text{O}$ than Antarctic ice, were formed in the latest Miocene. Evidence for the first mechanism is provided by detailed biostratigraphical studies which reveal a hiatus in nearly all latest Miocene

sediment recovered from the Pacific Ocean by the Deep Sea Drilling Project²⁷. The second mechanism is supported by Mercer and Sutter²⁸ who suggest that a Northern Hemisphere ice-sheet about one-half the size of the Pleistocene Laurentide ice-sheet formed in the latest Miocene. The formation of this ice-sheet with an isotopic composition of $\sim -30\%$ could significantly lower sea level without dramatically raising the $\delta^{18}\text{O}$ of the oceans.

An understanding of the structure of the water column in the Betic area before the eustatic lowering of sea level and the Messinian evaporitic events allows us to place constraints on the stratigraphical position of the Caliza Tosca. Studies by Cita *et al.*²⁹ and Hsü *et al.*³⁰ indicate that the changes from open marine conditions occurred suddenly ($<10^4$ yr). There is no evidence in the Carmona section for a rapid increase in salinity to hypersaline conditions before the sea level fall. There is, however, a small ($<0.5\%$) gradual increase in bottom water $\delta^{18}\text{O}$ values up the section which possibly occurred as a result of the slow tectonic uplift of the Betic straits region gradually restricting bottom water return flow to the Atlantic. Thus, a eustatic lowering of sea level cut off the return flow to the Atlantic of deep waters resulting in rapid isolation of the Mediterranean Sea and causing hypersaline conditions.

We tentatively correlate the rapid lowering of sea level at Carmona with the beginning of hypersaline conditions within the Mediterranean region. A change to hypersaline conditions in the deep waters of the Mediterranean would have occurred rapidly as a result of surface water evaporation once the bottom water return flow to the Atlantic was shut off in the Betic straits. Initially, a restricted supply of North Atlantic surface waters maintained normal marine surface water salinities in the Mediterranean but eventually as the Atlantic portal closed the surface waters also became hypersaline. Total evaporation could have occurred within $\sim 10^3$ yr of final closure of the Atlantic-Mediterranean gateways. Thus, the change to hypersaline conditions in the Mediterranean probably occurred within at most, a few thousand years, of the eustatic sea-level event recorded at Carmona and therefore provides a valuable stratigraphical marker within the Mediterranean region independent of biostratigraphical criteria. Hypersaline conditions are recorded in the Tripoli (marly diatomites) of the Falconara section in Sicily^{31,32}, situated very near the Messinian stratotype sections at Pasquasia and Capodarso. Planktonic foraminiferal³¹ and bulk carbonate³² $\delta^{18}\text{O}$ values increase dramatically in the upper part of the Falconara section at the base of the Tripoli formation immediately following the demise of most benthic foraminifera, which suggests that the bottom waters became hypersaline before the surface waters.

We suggest that a eustatic sea-level fall recorded at Carmona and the global decrease in $\delta^{13}\text{C}$ values correlate with the lowermost Tripoli Formation based on the almost simultaneous (within 5,000 yr?) change to hypersaline conditions in the Falconara section. If we are correct in assigning an age of 6.2 Myr to the sea-level event and associated carbon isotope shift then the base of the Tripoli in the Falconara section is ~ 6.2 Myr. If the absence of *Nitzschia miocenica* and *Thalassiosira praeconvexa* is due to ecological exclusion³³, then the Tripoli could be as old as magnetic chron 6, in good agreement with our age estimate.

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1. Bender, M. L. & Keigwin, L. D. *Jr Earth planet. Sci. Lett.* **45**, 383–393 (1979).
2. Vincent, E., Killingley, J. S. & Berger, W. H. *Mar. Micropaleont.* **5**, 185–203 (1980).
3. Bender, M. L. & Graham, D. *Mar. Micropaleont.* **6**, 451–464 (1981).
4. Ingle, J. C. *Jr Init. Rep. DSDP Leg 18*, 949–960 (1973).
5. Bandy, O. L. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **5**, 63–75 (1967).
6. Berggren, W. A. & Haq, B. U. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **20**, 67–129 (1976).
7. Kennett, J. P. *Trans. R. Soc. N.Z.* **4**, 83–102 (1966); *N.Z. J. Geol. Geophys.* **10**, 1051–1063 (1967).

8. Hsü, K. J. *et al. Nature* **267**, 399–403 (1977).
9. Adams, C. G., Benson, R. H., Kidd, R. B., Ryan, W. B. F. & Wright, R. C. *Nature* **269**, 383–386 (1977).
10. Keigwin, L. D. *Jr Init. Rep. DSDP Leg 68*, 445–453 (1982).
11. Brewster, N. A. *Bull. geol. Soc. Am.* **91**, 337–347 (1980).
12. Leinen, M. *Bull. geol. Soc. Am.* **90**, 1310–1376 (1979).
13. Van Andel, T. J. H. *Earth planet. Sci. Lett.* **26**, 187–194 (1975).
14. Davies, T. A. & Worsley, T. R. in *DSDP—A Decade of Progress: SEPM Vol.* 169–179 (1981).
15. Broecker, W. S. *Quat. Res.* **1**, 188–207 (1971); in *Climatic Variations and Variability: Facts and Theories*, 111–121 (Reidel, Dordrecht, 1981).
16. Vail, P. R. & Hardenbol, J. *Oceanus* **22**, 71–79 (1979).
17. Keigwin, L. D. *Jr Earth planet. Sci. Lett.* **45**, 361–382 (1979).
18. Loutit, T. S. & Kennett, J. P. *Science* **204**, 1196–1199 (1979).
19. Keigwin, L. D. Jr & Shackleton, J. J. *Nature* **284**, 613–614 (1980).
20. Haq, B. U. *et al. Geology* **18**, 427–431 (1980).
21. Benson, R. H. *Init. Rep. DSDP Leg 42*, 777–788 (1978).
22. Grazzini, C. V. in *Init. Rep. DSDP Leg 42*, 829–836 (1978).
23. Carter, A. N. *Nature* **276**, 258–259 (1978).
24. Shackleton, N. J. & Kennett, J. P. *Init. Rep. DSDP Leg 29*, 801–807 (1975).
25. Loutit, T. S. *Mar. Micropaleont.* **6**, 1–27 (1981).
26. Woodruff, F., Savin, S. M. & Douglas, R. G. *Science* **212**, 665–668 (1981).
27. Keller, G. & Barron, J. A. *Bull. geol. Soc. Am.* (submitted).
28. Mercer, J. H. & Sutter, J. F. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **38**, 185–206 (1982).
29. Cita, M. B., Wright, R. C., Ryan, W. B. F. & Longinelli, A. *Init. Rep. DSDP Leg 42*, 1003–1036 (1978).
30. Hsü, K. J. *et al. Init. Rep. DSDP Leg 42*, (1978).
31. Van der Zwaan, G. J. *Proc. of the Koninklijke Nederlandse Akademie van Wetenschappen, Ser. B* **82**, 487–502 (1979).
32. McKenzie, J. A., Jenkyns, H. C. & Bennet, G. G. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **29**, 125–141 (1979).
33. Burckle, L. D. *Abstr. 2nd Messinian Conf.*, Gargagno **43** (1976).

Isotopic and geochemical studies of nodules in kimberlite have implications for the lower continental crust

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Lower crustal xenoliths from the Calcutteroo kimberlitic pipes in South Australia show a surprisingly diverse range in Nd and Sr isotopic compositions and rare earth element (REE) abundances. Mafic granulite and garnet-clinopyroxenite xenoliths have measured $\epsilon_{\text{Nd}}(0)$ values between +6.1 and -7.9, both light (L)REE enrichments and depletions, low Rb/Sr ratios (0.005–0.040) and relatively high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of from 0.70675 to 0.70919. A felsic xenolith has compositions more typical of upper crustal rocks with $\epsilon_{\text{Nd}}(0) = -21.4$, LREE enrichments, Rb/Sr = 1.47 and $^{87}\text{Sr}/^{86}\text{Sr} = 0.85987$. An approximate correlation also exists between Sm/Nd versus $^{143}\text{Nd}/^{144}\text{Nd}$ and Rb/Sr versus $^{87}\text{Sr}/^{86}\text{Sr}$ indicating major intra-crustal differentiation in the lower crust at $\sim 2,400$ Myr. This produced extremely low Rb/Sr ratios, and both relative LREE enrichments (low Sm/Nd) and depletions (high Sm/Nd). If these results are typical, then they indicate that the lower crust has higher $^{87}\text{Sr}/^{86}\text{Sr}$ and more positive $\epsilon_{\text{Nd}}(0)$ values than previous estimates^{1–5}. This may require revision of total crustal compositions and crust-mantle evolutionary models^{1–5}.

Although the composition of the upper continental crust is established reasonably well by direct observations, the composition of the lower crust remains uncertain. Xenoliths derived from lower crustal depths by kimberlite magmas provide one of the few direct means of sampling the lower crust, as it exists *in situ* at present. In this study, we present Nd and Sr isotopic data, together with selected trace element abundances for lower crustal xenoliths from the Calcutteroo kimberlitic pipes in South Australia. The kimberlite host rocks of the inclusions are intruded into folded, Proterozoic marine sediments⁶, located in the Flinders Range, about 230 km north of Adelaide in South Australia. Measured Rb/Sr ages on phlogopites separated from kimberlitic matrices are in the range 164–174 Myr (ref. 7).

The inclusions consist of garnet clinopyroxenites, garnet granulites and felsic garnetiferous gneisses. Grain sizes vary

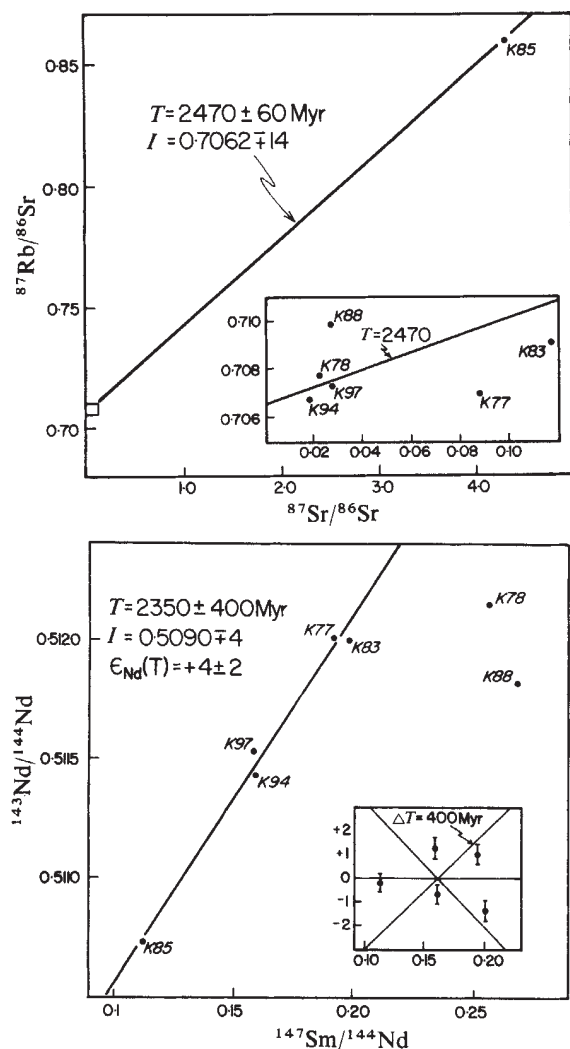


Fig. 1 *a*, Rb-Sr isochron diagram for lower crustal xenoliths from south Australia. The age is controlled by the felsic granulite K85. The low Rb/Sr samples (shown in inset) have significant deviations from the 2,470 Myr isochron but all indicate high initial $^{87}\text{Sr}/^{86}\text{Sr}$. *b*, Sm-Nd isochron diagram. A regression (excluding K78, K88) gives a similar age to Rb-Sr. Inset shows deviations from the best fit isochron in parts per 10^4 . The deviant samples K78 and K88 indicate that the lower crust sampled by the kimberlite is heterogeneous in age and/or initial ratios.

from fairly uniform, microgranular (<0.5 mm) through sub-equant granular (0.5–1.5 mm) to porphyroblastic (<5.0 mm garnet). Some samples have a distinct mineral lineation. Phase assemblages in the basic inclusions are dominated by omphacitic diopside-salite, intermediate Mg/Fe garnet with ~20–25 mol % grossular, and plagioclase in the compositional range oligoclase-andesine (R.J.A., J.F. and B.W.C., in preparation). Minor and accessory phases consist of rutile, biotite, hastingsitic amphibole, K-rich feldspar, scapolite, quartz, apatite and sphene. The more felsic nodules have a diverse mineralogy including, in the sample analysed for Nd and Sr isotopes, almandine-rich garnet, K-rich feldspar (Ca:Na:K ≈ 0.5:19:80.5), kyanite, quartz, rutile and biotite (Mg/Mg + ΣFe ≈ 0.62). Orthopyroxene, amphibole, sphene, scapolite and rare REE-U-rich phosphate phases are present in variable proportions in other inclusions of intermediate to felsic composition in the Calcutteroo kimberlitic pipes⁸ (R.J.A. *et al.*, in preparation). Equilibration temperatures and pressures^{9–12} for the inclusions are in the range 800–900°C, 8–12 kbar equivalent to depths in the crust of the order 25–40 km. The depth of the Moho in this region is suggested to be ~35 km (ref. 13). Most inclusions are basaltic in chemistry with <51%

SiO₂, and MgO in the range 7–11 wt%. Intermediate SiO₂ (~55%) and high-SiO₂ (60–68%) inclusions constitute a minor fraction of the inclusion population.

The Sm-Nd and Rb-Sr isotopic measurements are given in Table 1 and shown in Fig. 1*a, b*. There are correlations between measured Sm/Nd versus $^{143}\text{Nd}/^{144}\text{Nd}$ and Rb/Sr versus $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (Fig. 1*a, b*). A regression of all the Rb-Sr isotopic data yields an age of $2,470 \pm 60$ Myr and an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7062 ± 14 . The age is controlled solely by the high Rb/Sr sample (K85) and the initial $^{87}\text{Sr}/^{86}\text{Sr}$ by the remaining low Rb/Sr samples. A regression of the Sm-Nd isotopic data (excluding K78 and K88) yields a similar, although less precise, age of $2,350 \pm 400$ Myr and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.5090 ± 4 (corresponding to $\epsilon_{\text{Nd}}(T) = +4 \pm 2$). The xenoliths K78 and K88 deviate markedly from this array. These latter samples also have pronounced LREE depletions (Fig. 2) thus excluding the possibility that their REE abundances and Nd isotopic compositions have been modified by contamination with the host kimberlite magma, which is strongly enriched in LREE⁸. Instead, the deviations must reflect some real heterogeneities in age and/or initial ratios of the lower crust sampled by the xenoliths.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7062 ± 14 is substantially greater than mantle values indicating that the major chemical fractionation event registered by both isotopic systems occurred within the crust rather than as a consequence of mantle-crust differentiation processes. The high initial Sr ratio can be accounted for by a period before ~2,400 Myr of high Rb/Sr ratios within the lower crustal protoliths. For example, with an Rb/Sr ratio the same as K85 (Rb/Sr = 1.5), $^{87}\text{Sr}/^{86}\text{Sr}$ would evolve in an interval of ~80 Myr from a mantle value of 0.702 to the observed initial ratio of 0.7062. For a Rb/Sr ratio more typical of the upper crust (Rb/Sr = 0.3)¹⁴, this interval would be increased to ~400 Myr. The initial Nd isotopic composition of $\epsilon_{\text{Nd}}(T) = +4 \pm 2$ indicates derivation from a source which has had a previous history of relative LREE depletion. Although this is generally more characteristic of mantle sources^{3,4,15,16}, it is still compatible with a relatively short crustal residence time. For example, assuming that the initial crust was LREE enriched (similar to K85), a crustal prehistory of ~100 Myr would reduce the $\epsilon_{\text{Nd}}(T)$ value by ~1 unit. Therefore, to account for the observed ϵ_{Nd} (2,400 Myr) value of $+4 \pm 2$, this would imply derivation from a mantle source with ϵ_{Nd} (2,500 Myr) = $+5 \pm 2$, which is still compatible with available constraints¹⁶. Substantially longer crustal prehistories are possible if the lower crustal protoliths had either chondritic LREE or depleted LREE abundances. This is conceivable considering the highly variable REE abundances in the xenoliths.

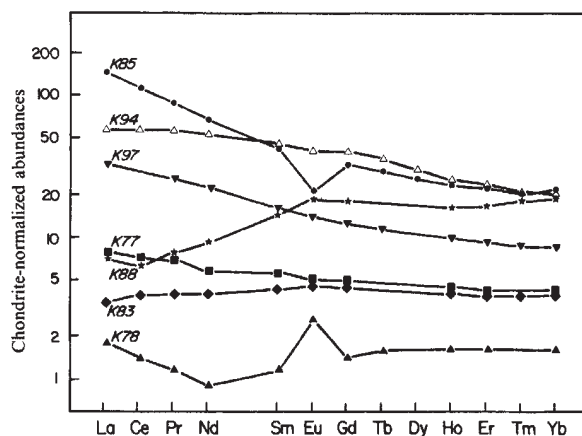


Fig. 2 REE patterns determined using spark-source mass spectrometry²⁴ and normalized to chondritic abundances. The lower crustal xenoliths have a diversity of patterns and only K78 has the positive Eu anomaly predicted for the lower crust^{14,17} and found in the Lesotho granulite xenoliths²⁵.

Table 1 South Australian lower crustal xenoliths

Samples	Sm	Nd	Rb	Sr	$^{87}\text{Rb}/^{86}\text{Sr}$	$^{147}\text{Sm}/^{144}\text{Nd}$	$^{143}\text{Nd}/^{144}\text{Nd}^\dagger$	$^{87}\text{Sr}/^{86}\text{Sr}^\dagger$	$\epsilon_{\text{Nd}}(0)^\ddagger$
K77 gnt-cpx-plag (bio-rut-qtz)*	1.05	3.32	4.16	137.3	0.0875	0.1900	0.51201 ± 3	0.70708 ± 6	3.4
K78 gnt-cpx-plag (bio-rut-qtz)	0.29	0.67	1.02	135.2	0.0218	0.2565	0.51215 ± 6	0.70770 ± 5	6.1
K83 gnt-cpx (sc-rut-qtz)	0.95	2.90	4.37	108.1	0.117	0.1979	0.51200 ± 2	0.70919 ± 6	3.2
K85 gnt-kyanite-kspar-qtz (bio-rut)	8.84	47.56	169	115	4.306	0.1124	0.51074 ± 2	0.85987 ± 6	-21.4
K88 gnt-cpx-plag (rut-apt-amph)	1.87	4.20	1.47	155.1	0.027	0.2694	0.51182 ± 3	0.70980 ± 8	-0.3
K94 gnt-cpx-plag (qtz-rut-apt)	9.59	36.52	1.34	215.0	0.018	0.1588	0.51143 ± 3	0.70675 ± 4	-7.9
K97 gnt-cpx-plag (qtz-rut-apt-sphene)	4.19	16.01	1.78	187.7	0.0275	0.1584	0.51153 ± 2	0.70736 ± 5	-6.0
§ Average South Australian lower crust	3.2	11.8	7.5	180	0.12	0.16	0.51179	0.7128	-1.0

* Major phase listed first, minor and accessory phases in parentheses. gnt, garnet; cpx, clinopyroxene; plag, plagioclase; bio, biotite; qtz, quartz; rut, rutile; amp, amphibole; apt, apatite; kspar, K-rich feldspar; sc, scapolite.

† Nd and Sr isotopic compositions and abundances determined using analytical procedures described in ref. 26. Normalized to $^{146}\text{Nd}/^{142}\text{Nd} = 0.636151$ and $^{86}\text{Sr}/^{88}\text{Sr} = 0.1194$.

‡ $\epsilon_{\text{Nd}}(0) = \left[\frac{(^{143}\text{Nd}/^{144}\text{Nd})_{\text{sample}}}{(^{143}\text{Nd}/^{144}\text{Nd})_{\text{CHUR}}} - 1 \right] \times 10^4$, where $(^{143}\text{Nd}/^{144}\text{Nd})_{\text{CHUR}} = 0.511836$.

§ Average values calculated by weighing xenoliths according to their relative abundance.

Intracrustal melting at ~2,500 Myr has also been proposed by Taylor¹⁷ and Taylor and McLennan¹⁴ to account for the negative Eu anomaly observed in post-Archaeon sediments. During this intracrustal melting event, it is envisaged that the melt becomes enriched in LREE and Rb, and depleted in Eu and Sr as a result of the preferential incorporation of Eu^{2+} and Sr^{2+} into residual plagioclase. Although this mechanism accounts for the low Rb and high Sr abundances, of the xenoliths, only one has a positive Eu anomaly. (In fact, K85 has a negative Eu anomaly.) Thus, at least for the samples analysed in this study, it would appear that retention of plagioclase in the lower crust has not been the major factor controlling the REE abundances. Variations in the abundance of REE enriched accessory minerals (such as allanite, zircon and apatite) could substantially influence the REE abundances in the lower crust, and perhaps compensate for the positive Eu anomaly produced by plagioclase.

The present-day Nd and Sr isotopic compositions are shown in Fig. 3, together with estimates of lower crustal compositions by other workers^{1-3,5,14}. The xenoliths analysed in this study clearly differ from all other lower crustal estimates, primarily due to the high measured $^{87}\text{Sr}/^{86}\text{Sr}$ ratios and more positive $\epsilon_{\text{Nd}}(0)$ values. Several workers have assumed that the relative REE abundances are the same in both the upper and lower crust^{1,2,4,18}. This assumption is based on the observation that Archaean felsic granulites usually have LREE-enriched patterns similar to upper crustal patterns¹⁸⁻²¹. Apart from the *ad hoc* assumption that Archaean felsic granulites are representative of the present day lower crust, the significance assigned to the felsic gneisses ignores the presence in many Archaean granulite terrains (such as Scourian^{5,20}) of granulites having intermediate, basic and ultramafic compositions. These latter rock types are all lower in absolute concentration of LREE, occasionally LREE-depleted, and have small positive or negative Eu anomalies. The relative proportions of these more basic rock types in these terrains is conjectural at present, but this study, as well as others on suites from kimberlitic pipes in New South Wales, Victoria, and the Colorado Plateau, USA, all suggest that basic rock types may be an important constituent of the lower crust^{8,22,23}.

Although reliable estimates of lower crustal compositions must await more data of the type reported here, it is, nevertheless, informative to speculate on its implications for total crustal compositions. Typical upper crust (for a mean age of 1,500

Myr)¹ has $\epsilon_{\text{Nd}}(0) = -15$ and $^{87}\text{Sr}/^{86}\text{Sr} = 0.7220$, Nd = 26 p.p.m. and Sr = 350 p.p.m. (ref. 14). Thus, total crust consisting of 1 part upper crust to 2 parts lower crust¹⁴ has $\epsilon_{\text{Nd}}(0) = -8.3$, $^{87}\text{Sr}/^{86}\text{Sr} = 0.7173$ (for a mean age of 1,500 Myr)¹ and Nd = 16 p.p.m. and Sr = 230 p.p.m. (based on our analyses of lower crustal xenoliths). These compositions are significantly different from previous estimates for the total crust and may require

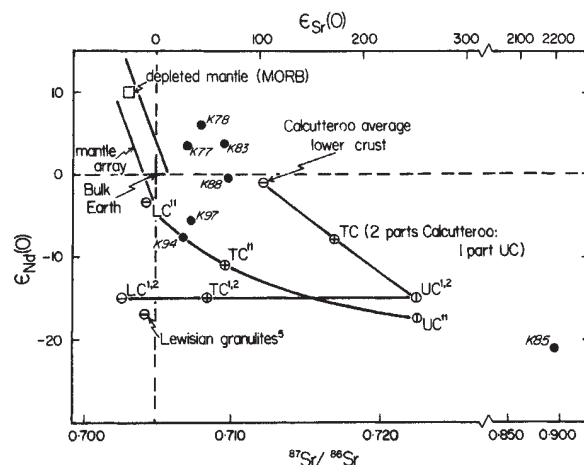


Fig. 3 Present-day Nd and Sr isotopic composition for south Australian lower crustal xenoliths (●). The xenoliths have both positive and negative $\epsilon_{\text{Nd}}(0)$ values and have relatively radiogenic $^{87}\text{Sr}/^{86}\text{Sr}$ ratios. The felsic granulite K85 which has extremely high $^{87}\text{Sr}/^{86}\text{Sr}$ and negative $\epsilon_{\text{Nd}}(0)$ values is a rare xenolith type. The south Australian lower crustal average is significantly different from existing models particularly those that assume that the relative LREE abundances are the same in the upper and lower crust^{1-5,18}. The closest agreement is with the models of Taylor¹⁷ and Taylor and McLennan¹⁴ which assume intracrustal fractionation of Sm/Nd as well as Rb/Sr and thus predict more positive $\epsilon_{\text{Nd}}(0)$ values for the lower crust compared with the upper crust. Lines connecting lower crust (LC ⊖), total crust (TC ⊕) and upper crust (UC ⊕) are calculated by assuming that the lower crust constitutes two-thirds of the total. The inferred total crust composition calculated using the lower crustal xenoliths is also substantially different from previous estimates^{1,2,14}. If this is not due to Ha sampling bias, it may imply, for example, the existence of additional reservoirs to satisfy the bulk Earth constraint¹⁻⁵ of $\epsilon_{\text{Sr}}(0) = \epsilon_{\text{Nd}}(0) = 0$.

revision of the parameters and assumptions on which some crust-mantle evolutionary models¹⁻⁵ are based. In particular, the assumption that the total crust and depleted mantle are strictly complementary and are together equivalent to the bulk Earth¹⁻⁵ is not consistent with these results.

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- Jacobsen, S. B. & Wasserburg, G. J. *J. geophys. Res.* **84**, 7411-7427 (1979).
- DePaolo, D. J. *Geochim. cosmochim. Acta* **44**, 1185-1196 (1980).
- O'Nions, R. K. & Hamilton, P. J. *Phil. Trans. R. Soc. A* **301**, 473-487 (1981).
- Allègre, C. J. *Tectonophysics* **81**, 109-132 (1982).
- Weaver, B. L. & Tarney, J. *Nature* **286**, 342-346 (1980).
- Colchester, D. M. *J. geol. Soc. Aust.* **19**, 383-386 (1972).
- Stracke, K. J., Ferguson, J. & Black, L. P. in *Proc. 2nd int. Kimberlite Conf.* **1**, 71-91 (1979).
- Ferguson, J., Arculus, R. J. & Joyce, J. *BMR J. Aust. Geol. Geophys.* **4**, 227-241 (1979).
- Perkins, D. III & Newton, R. C. *Nature* **292**, 144-146 (1981).
- Goldsmith, J. R. *Am. Miner.* **65**, 272-284 (1980).
- Ellis, D. J. & Green, D. H. *Contr. Miner. Petrol.* **71**, 13-22 (1979).
- Ganguly, J. *Geochim. cosmochim. Acta* **43**, 1021-1029 (1979).
- Branson, J. C., Moss, F. J. & Taylor, F. J. *Bur. Min. Res. Austr. Rep.* **183** (1976).
- Taylor, S. R. & McLennan, S. M. *Phil. Trans. R. Soc. A* **301**, 381-399 (1981).
- DePaolo, D. J. *EOS* **62**, 137-140 (1981).
- McCulloch, M. T. & Compston, W. *Nature* **294**, 322-327 (1981).
- Taylor, S. R. *A. Geophys. Un. Ewing Ser.* **1**, 325-335 (1977).
- DePaolo, D. J. & Wasserburg, G. J. *Geochim. cosmochim. Acta* **43**, 615-627 (1979).
- Green, T. H., Brunfelt, A. O. & Heier, K. S. *Geochim. cosmochim. Acta* **36**, 241-257 (1972).
- Muecke, G. K., Pride, C. & Sarkar, P. *Phys. Chem. Earth* **11**, 449-464 (1979).
- Tarney, J., Weaver, B. L. & Drury, S. A. in *Trondhjemites, Dacites and Related Rocks* (ed. Barker, F.) 275-299 (Elsevier, Amsterdam, 1979).
- Arculus, R. J. & Smith, D. in *Proc. 2nd int. Kimberlite Conf.* **2**, 309-317 (1979).
- McGetchin, T. R. & Silver, L. T. *J. geophys. Res.* **77**, 7022-7037 (1972).
- Taylor, S. R. & Gorton, M. P. *Geochim. cosmochim. Acta* **41**, 1375-1380 (1977).
- Rogers, N. W. *Nature* **270**, 681-684 (1977).
- McCulloch, M. T. & Chappell, B. W. *Earth planet. Sci. Lett.* **58**, 51-64 (1982).

Ages and implications of East Pacific Rise sulphide deposits at 21°N

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Massive sulphide deposits described as inactive hydrothermal deposits (samples CYP) along the axis of East Pacific Rise (EPR) were first discovered at 21°N, about 700 m west of the active rift¹. This discovery was followed by that of active vents made of sulphides (samples *Alvin*) in the central part of the rift valley². Due to the importance of the flux of elements presently discharging and its impact on the chemistry of the ocean, it is important to understand the duration of the phenomenon and its possible periodicity. We present here an attempt to date the fossil and the active chimneys using radioisotope methods.

In the oceanic environment, chemical fractionations exist due to differences in geochemical behaviour of the different nuclides forming the decay series of uranium and thorium. Their existence in the hydrothermal environment can be postulated.

(1) The chemical separation between uranium and thorium is well known in normal oceanic conditions, and ²³⁰Th has been widely used as a chronometer for oceanic events³⁻⁷. This method is applicable in the range of some thousands of years to about 300,000 yr. This time span cannot be used for the active vents but may be of interest for the fossil chimneys as the age of the crust on which they lie, at 700 m from the active axis, may be estimated taking into account a mean half expansion rate of 3 cm yr⁻¹ (ref. 8) as ~20,000 yr (ref. 1).

Uranium and thorium isotopes have been measured by α -ray spectrometry after chemical separation; results are given in Table 1. To ascertain that no ²³⁰Th enters in the sulphides at the moment of the precipitation, on 10 samples from active vents, we have checked that ²³⁰Th is always under the detection limit of the apparatus (Table 2), as well as ²³²Th, so the latter may be used to detect an eventual contamination during alteration.

On two samples from the fossil deposits, CYP 78-08-14 and CYP 78-12-40, we have analysed 11 different subsamples, 8 of them are pure sulphides, they do not contain any ²³²Th and their ²³⁰Th/²³⁴U ages are from <2,700 to 6,400 yr (ref. 9), (Table 1). Three subsamples which are partly oxidized exhibit measurable concentrations of ²³²Th and higher uranium contents. The calculated ages for these samples are 6,900, 18,000 and 36,500 yr. Although the first two do not disagree with the possible age of the basement, we discard them as being wrong due to their probable alteration. Thus, we conclude that a former period of active hydrothermal discharge took place ~4,000 yr ago.

(2) The half life of ²³⁰Th, 75,200 yr, precludes its use for dating the chimneys sampled during *Alvin* dives in the central part of the rift valley, so we tried to use different short-lived decay products of the uranium and thorium series.

Turekian *et al.*¹⁰ have used, for the measurement of growth rates of giant clams from Galapagos rise, the ratios ²²⁸Th/²²⁸Ra; ²²⁸Ra/²²⁶Ra and ²¹⁰Pb/²²⁶Ra. Those nuclides have half lives between 1,622 and 1.90 yr which seem suitable for our study, provided we know the ratios at the origin of the system. Moreover, we also tried to use the decrease of activity of ²¹⁰Pb ($t_{1/2} = 22$ yr), referred to stable lead.

The activities of these nuclides have been measured by non-destructive γ -ray spectrometry, and lead was measured by atomic absorption spectrophotometry.

The ²¹⁰Pb activity has been measured in the particulate sulphides precipitating directly in the plume of an hydrothermal vent as 1.8 d.p.m. ²¹⁰Pb μ g⁻¹ of Pb (ref. 11). We consider that the lead in the sulphides constituting the walls of the vents has

Table 1 U and Th isotopic analysis by α -ray spectrometry

Sample	²³² Th (p.p.m.)	²³⁸ U (p.p.m.)	²³⁴ U/ ²³⁸ U (activities ratio)	²³⁰ Th (d.p.m. g ⁻¹)	²³⁰ Th/ ²³⁴ U (activities ratio)	Age (kyr)
CYP 78-08-14						
A-1	0.09 ± 0.06	14.4 ± 0.4	1.159 ± 0.023	0.75 ± 0.03	0.061 ± 0.003	6.9 ± 0.35
A-2 (1a)	0.40 ± 0.09	13.1 ± 0.5	1.139 ± 0.037	1.7 ± 0.1	0.153 ± 0.011	18.0 ± 1.4
A-2 (1b)	0.21 ± 0.04	8.9 ± 0.3	1.131 ± 0.024	2.14 ± 0.09	0.287 ± 0.015	36.5 ± 2.3
A-2 _{II}						
External layer 1	≤ 0.02	1.75 ± 0.06	1.10 ± 0.05	0.031 ± 0.007	0.022 ± 0.006	2.4 ± 0.7
Layer 2	≤ 0.03	1.19 ± 0.06	1.174 ± 0.067	0.035 ± 0.010	0.034 ± 0.011	3.8 ± 1.2
Layer 3	n.d.	1.31 ± 0.06	1.072 ± 0.068	0.041 ± 0.018	0.039 ± 0.019	4.3 ± 2.1
Internal layer 4	≤ 0.016	3.0 ± 0.1	1.123 ± 0.034	0.064 ± 0.007	0.026 ± 0.004	2.9 ± 0.5
CYP 78-12-40						
A	≤ 0.02	1.22 ± 0.05	1.192 ± 0.059	0.031 ± 0.008	0.029 ± 0.008	3.2 ± 0.9
B External pustules	≤ 0.04	0.61 ± 0.04	1.1 ± 0.1	≤ 0.012	≤ 0.024	≤ 2.7
Intermediate part	≤ 0.016	1.58 ± 0.05	1.082 ± 0.038	0.050 ± 0.006	0.040 ± 0.006	4.4 ± 0.6
Internal part	≤ 0.08	2.8 ± 0.1	1.104 ± 0.039	0.081 ± 0.031	0.035 ± 0.014	3.9 ± 1.6

Errors quoted are 1 σ uncertainties based on counting statistics.

Table 2 U, Th and Pb isotopic analysis

Dive	Sample	Subsample	^{230}Th (d.p.m. g ⁻¹)	^{234}U (d.p.m. g ⁻¹)	^{238}U (p.p.m.)	Pb (p.p.m.)	^{210}Pb (d.p.m. g ⁻¹) whole sample	$^{210}\text{Pb}/\text{Pb}$ (d.p.m. µg ⁻¹)	Age (yr)
<i>Alvin 1</i>									
909	R1	A	≤0.005	3.00±0.16	3.4±0.2	910	600±105	0.66±0.11	32±6
914	R1	A	≤0.006	3.37±0.16	4.0±0.2	495	213±36	0.43±0.07	46±5
923	R7	E	≤0.006	4.63±0.16	5.3±0.2	850	281±38	0.33±0.05	54±4
<i>Alvin 2</i>									
978	R13	1	≤0.006	0.32±0.02	0.34±0.03	330	259±37	0.78±0.11	27±5
979	R3	1	≤0.006	0.82±0.05	1.01±0.06	3,700	1000±116	0.27±0.03	61±4
980	R5	1	≤0.005	1.15±0.05	1.45±0.07	690	360±50	0.52±0.07	40±4
981	R3	1	≤0.005	0.080±0.006	0.085±0.006	240	103±32	0.43±0.13	46±10
982	R21	1.1	≤0.006	4.16±0.21	5.1±0.3	660	519±70	0.79±0.10	26±4
		1.3	≤0.005	1.10±0.06	1.28±0.07	160	139±42	0.87±0.25	23±10
982	Chimney		≤0.004	0.38±0.02	0.48±0.02	≤60	27±24	—	—
<i>Cyana</i>									
CYP78.12	40B		0.050±0.006	1.26±0.04	1.58±0.05	390	28±21		

$^{230}\text{Th}/^{234}\text{U}$ ages have not been calculated as they have no significance. The error on γ measurements of ^{210}Pb is 10 statistical errors on counting rate + an error on the efficacy of Ge-Li detector estimated as 10%. The error on atomic absorption measurement of Pb is ~5% and has been neglected.

the same origin and the same ^{210}Pb activity at the time of deposition. On nine samples, ^{210}Pb activities were large enough to allow relatively precise measurements even by γ -ray spectrometry, giving $^{210}\text{Pb}/\text{Pb}$ ratios from 0.87 ± 0.25 to 0.27 ± 0.03 (Table 2)¹².

In ambient seawater, some Pb and ^{210}Pb may exist, 1 ng per kg (ref. 13) and 20 d.p.m. per 100 kg (ref. 11) respectively; this oceanic component might change the initial $^{210}\text{Pb}/\text{Pb}$ ratio and also progressively contaminate the sulphides after their formation. We therefore verified that, in one of the 'old' samples (CYP78-12-40B) containing stable lead, ^{210}Pb activity was below the limit of our method (28 ± 21 d.p.m. g⁻¹) and that on a presently forming sulphide deposit containing almost no Pb (982 chimney), ^{210}Pb is not measurable. So, unless the initial $^{210}\text{Pb}/\text{Pb}$ ratio measured by Finkel *et al.*¹¹ varies from one vent to another in the same field, or if it is different in the sulphides deposited at the vent from the initial one, which is unlikely, the ages so obtained are true ages.

Unfortunately, the samples coming from different vents were not sufficiently documented especially as regards their orientation and position in the walls of the vents, to allow the meaning of the differences in ages to be discussed. Either they form continuously and our values are mean values between an older part and a very recent one, and, in this case, it is a lower limit for the beginning of the event, or the chimneys took only a brief time to build and do not grow at present, and our age is the age of the beginning of the event. During the last *Cyathern* cruise, the instantaneous growth of a chimney has been visualized (R. Hekinian, personal communication) which suggests that our second hypothesis is more valid.

$^{228}\text{Th}/^{228}\text{Ra}$ method may be applied to samples containing radium. As they do not contain any thorium when they formed, then any measurable ^{228}Th must have formed as a decay product of ^{228}Ra . Due to the relatively short half lives of the two nuclides, the equilibrium value of the activity ratio, 1.4, is

obtained after about 15 yr. During this time, ^{228}Ra being unsupported decreases with its half life of 5.75 yr while ^{228}Th increases with its half life of 1.9 yr.

Only two of our samples exhibited ^{228}Th and ^{228}Ra activities measurable by γ -ray spectrometry (Table 3) for which the equilibrium value of the ratio is obtained, giving for the two samples ages >15 yr, which is in accordance with the 46 ± 5 and 54 ± 4 yr found with ^{210}Pb .

The last suitable ratio is $^{228}\text{Ra}/^{226}\text{Ra}$. In such a young system, ^{226}Ra may be considered as stable while ^{228}Ra decreases. The problem is then to know exactly the initial value of the ratio. We may hypothesize that the two nuclides are in equilibrium with their parents ^{238}U and ^{232}Th respectively in the basalt and that they are not differentiated during leaching and precipitation. These hypotheses are quite reasonable as there is no reason to have an isotopic fractionation. The problem is then to know the $^{232}\text{Th}/^{238}\text{U}$ ratio of the basalts. The weight ratio is given as 2 for mid-oceanic ridge basalts, on average¹⁴, which gives an activity ratio of 0.65. For the only two samples in which radium is present (Table 3) the measured ratios lead to ages of 16 and 12 yr, lower than the ages found with ^{210}Pb .

Recent measurements¹⁵ have shown that tholeiitic basalts from 21° N EPR have a higher ^{232}Th content, leading to a mean activity ratio of 1.13, the ages so obtained are respectively 21 and 17 yr. Moreover, in young basaltic rocks, the uranium series may be out of equilibrium, particularly ^{230}Th may be depleted which would imply a higher initial $^{228}\text{Ra}/^{226}\text{Ra}$ ratio. Anyway, these ages are too young compared with ^{210}Pb ages. But, due to the uncertainties in the initial $^{228}\text{Ra}/^{226}\text{Ra}$, to the lack of sensitivity of γ rays measurements and to the possible higher mobility of radium isotopes than of lead isotopes, we prefer, until new measurements on well documented samples are obtained, to consider that ^{210}Pb ages are the most probable. It must be pointed out that such low ^{226}Ra activity compared with ^{210}Pb activity have no appreciable influence on $^{210}\text{Pb}/\text{Pb}$ ages.

Table 3 Th and Ra isotopic analyses by γ -ray spectrometry

Sample	^{226}Ra (d.p.m. g ⁻¹)	^{228}Ra (d.p.m. g ⁻¹)	^{228}Th (d.p.m. g ⁻¹)	$^{228}\text{Th}/^{228}\text{Ra}$	$^{228}\text{Ra}/^{226}\text{Ra}$
<i>Alvin 1</i>					
914 R1 A	46±4.7	4.2±0.7	6.2±0.9	1.48±0.32	0.091±0.018
923 R7 E	15±1.6	2.3±0.5	3.8±0.7	1.65±0.50	0.15±0.04

Error is 1 σ statistical error on counting rate + an error on the efficacy of Ge-Li detector estimated equal to 10%. ^{226}Ra has been measured through ^{214}Bi ; ^{228}Ra has been measured through ^{228}Ac ; ^{228}Th has been measured through ^{208}Tl .

We conclude that: (1) These dating methods work and their precision may be improved using radiochemical separation followed by α and β countings. (2) There are two well defined systems, one which formed about 4,000 yr ago and another which is active at present. If such systems cannot develop far from the active axis, it shows that during the past 4,000 yr, the spreading rate has been as high as 15 cm yr^{-1} , and not 3 cm yr^{-1} . (3) The active system probably lasts $<100 \text{ yr}$, but $>20 \text{ yr}$, which is in relatively good accordance with the values calculated in ref. 16 from heat flow theoretical calculations.

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- Francheteau, J. *et al.* *Nature* **277**, 523–528 (1979).
- Rise Project Group *Science* **207**, 1421–1442 (1980).
- Barnes, J. W., Lang, E. J. & Potratz, H. A. *Science* **124**, 175–176 (1956).
- Ku, T. L. & Broecker, W. S. *Deep Sea Res.* **16**, 625–637 (1969).
- Scott, M. R., Scott, R. B., Rona, P. A., Butler, L. W. & Nalwak, A. J. *Geophys. Res. Lett.* **1**, 8, 355–358 (1974).
- Moore, W. S. & Vogt, P. R. *Earth planet. Sci. Lett.* **29**, 349–356 (1976).
- Lalou, C., Brichet, E., Ku, T. L. & Jehanno, C. *Mar. Geol.* **24**, 245–258 (1977).
- Larson, R. L., Menard, H. W. & Smith, S. M. *Science* **161**, 781–784 (1968).
- Lalou, C. & Brichet, E. *C. r. hebdom. Séanc. Acad. Sci., Paris* **290**, 819–822 (1980).
- Turekian, K. K., Cochran, J. K. & Nozaki, Y. *Nature* **280**, 385–387 (1979).
- Finkel, R. C., Macdougall, J. D. & Chung, Y. C. *Geophys. Res. Lett.* **7**, (9), 685–688 (1980).
- Lalou, C. & Brichet, E. *C. r. hebdom. Séanc. Acad. Sci., Paris* **293**, 821–826 (1981).
- Schaule, B. & Patterson, C. *Proc. int. Expert Discussion on Lead* (Pergamon, Oxford, 1978).
- Tatsumoto, M. *Earth planet. Sci. Lett.* **38**, 63–87 (1978).
- Vidal, Ph & Clauer, N. *Earth planet. Sci. Lett.* **55**, 237–246 (1981).
- Macdonald, K. C., Becker, K., Spiess, F. N. & Ballard, R. D. *Earth planet. Sci. Lett.* **48**, 1–7 (1980).

Carbon geodynamic cycle

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The systematic use of coupled isotopic tracers has improved our understanding of many problems such as the geochemical structure and evolution of the mantle. However, some fundamental questions remain^{1–6}; for example, does sediment injection occur in the mantle^{4,5,7}, modifying composition and combining the internal and external geochemical cycles? Recently ³He data from the South Atlantic have been used to suggest that the sediment injection phenomenon may be important on a local scale⁸. Here, using carbon data only, we show that sediment injection in the mantle must occur. This conclusion was reached after comparison between the integrated flux coming from the mantle and the budget of the exogeneous reservoir of carbon.

The value of the isotopic composition of carbon in the mantle was confusing for a long time. Carbonatites and most diamonds yielded values of $\delta^{13}\text{C}$ between -9% and -2% PDB (refs 9–12) whereas basalts yielded values between -25% and -30% (refs 12–14) (Fig. 1). Although they exist for a variety of acid residues from continental alkali rocks down to -30% (ref. 13), we are not aware of $\delta^{13}\text{C}$ lower than -26.5% for MORB tholeiites¹⁵. The difference between these values is of the same order of magnitude as that between reduced and oxidized forms of carbon (coal and carbonates) present at the Earth's surface—in low temperature conditions where carbon isotopic fractionation is important¹⁶. The results for basalts are rather surprising considering that they are formed at high temperature and could

imply that the $^{13}\text{C}/^{12}\text{C}$ of the mantle is very heterogeneous. This situation has recently been clarified^{14,15,17}. The CO_2 -liquid melt carbon isotopic fractionation has been measured experimentally¹⁵ and varies from 4.5 to 4% between 1,180 and 1,280 °C. Thus, if a basalt of initial $\delta^{13}\text{C}$ around -7% reaches the surface after losing CO_2 , the latter is enriched in the heavy isotope and the basalt contains residual carbon of much lower $\delta^{13}\text{C}$.

Both the carbon isotope compositions of several fractions of oceanic tholeiites¹⁵ and of the peculiar types of tholeiites forming the popping rocks dredged on the Atlantic ridge¹⁴ are compatible with this mechanism.

The quantitative model suggested for this outgassing is based on the calculation of the fraction of degassed carbon (relative to the original amount). Using a Rayleigh^{14,15} distillation law, a relation between the isotopic compositions of the residual basalt (δ) and of the initial mantle (δ_0) can be written as

$$\delta - \delta_0 = 1,000(\alpha - 1) \ln f$$

with α the fractionation factor and f the carbon fraction remaining in the basalt.

Using the experimental values of $\delta_0 = -7\%$ and $1,000(\alpha - 1) = 4.5$ one may calculate a series of values for f corresponding to the different values of $\delta^{13}\text{C}$ for basalts. The commonly accepted value of $\delta^{13}\text{C} = -26\%$ corresponds to $f = 0.014$. With carbon contents of the rocks between 100 and 150 p.p.m. (ref. 14), this corresponds to an initial content of 6,800–10,200 p.p.m. In fact, $\delta^{13}\text{C}$ of residual carbon are not necessarily as low as -26% and distillation effects do not necessarily follow a Rayleigh model. A greater range of initial contents, of 2,000–10,000 p.p.m., is indicated by recent results¹⁸.

One may now look at the rate of degassing per year. The thickness of the basalt layer in the oceanic crust is 2 km whereas the total thickness of the crust (basalt + gabbro) which was initially liquid, is 5 km. One may consider either that all the liquid has been degassed, directly or through intense hydrothermal circulation at ridges, or only the basaltic layer. This corresponds to two extreme hypotheses.

Considering a constant average spreading rate of 4 cm yr^{-1} , we get:

$6 \times 10^9 \times$	2×10^5	0.01	7.2×10^{14}
$\frac{\text{cm}}{\text{cm yr}^{-1}}$	$\frac{\text{cm}}{\text{cm}}$	$\frac{\text{g yr}^{-1}}{\text{g yr}^{-1}}$	$\frac{\text{g yr}^{-1}}{\text{g yr}^{-1}}$
(4×2)	or	0.002	$=$
$\times$	5×10^5	$\times$	0.58×10^{14}
$\times$	$\frac{\text{cm}}{\text{cm}}$	$\times$	$\frac{\text{g cm}^{-3}}{\text{g cm}^{-3}}$
$\times$	3	$\times$	$\times$
$\times$	$\frac{\text{g cm}^{-3}}{\text{g cm}^{-3}}$	$\times$	$\times$
(Length (Spreading) of ridge)	(Thickness of material involved)	(Density of basalt)	(Carbon concentration degassed)

The total mass of sediments at the Earth's surface is $32 \times 10^{23} \text{ g}$, 15% of which are carbonates. This amount of carbonates corresponds to $5.75 \times 10^{22} \text{ g}$ of carbon^{16,17}.

The mass of the continental crust is $2.4 \times 10^{25} \text{ g}$ (ref. 19). Considering a complete granito-gneissic composition and using an average content of 300 p.p.m. carbon for these rocks²⁰, the granito-gneissic continental crust corresponds to $7.2 \times 10^{21} \text{ g}$ which is one hundredth of that present in the sediments and is thus negligible. However, considering the existence of metamorphic carbonated series which are omitted from this calculation, we must add $\sim 1 \times 10^{22} \text{ g}$.

The maximum total carbon now present at the Earth's surface is $8 \times 10^{22} \text{ g}$ (the amount of carbon in the atmosphere and the oceans is negligible in this context). This gives lifetimes of carbon in the external cycle of $1.1\text{--}14 \times 10^8 \text{ yr}$. (External is the cycle outside the solid Earth and includes hydrosphere, atmosphere, lithosphere and sediment, but internal includes cycles inside the Earth.) However, the extreme values which cumulate the uncertainties on initial contents and thickness of

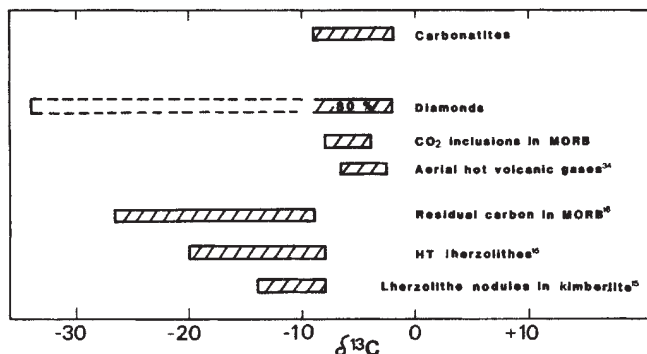


Fig. 1 The different ranges of values of $\delta^{13}\text{C}$ for various mantle materials.

$$\delta^{13}\text{C} = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{standard}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} \times 10^3$$

Standard is the PDB limestone. Results are from refs 12, 15, 18, 34, 35.

material involved are probably over-pessimistic, and the use of the mean rate value ($3.9 \times 10^{14} \text{ g yr}^{-1}$) which gives a lifetime of $2 \times 10^8 \text{ yr}$ is probably more realistic. Another way of expressing this result is to measure how much carbon has been degassed during $4 \times 10^9 \text{ yr}$ thus gaining information on the internal history of the Earth. Using a steady-state hypothesis, we get a mean value of 1.8×10^{24} , which represents 22 times the carbon now contained in the external parts of the planet.

We have considered a steady-state regime for the degassing during geological times but specialists agree that the latter decreased following weakening of the convection throughout this period.

One way of reconciling our observations is to admit that the exogenous cycle does not represent a reservoir of accumulation but of exchange. The carbon would thus be injected in the mantle at subduction zones, within the sediments.

Many objections have been made to the idea. How could light and soft sediments sink into a dense, hard and hot mantle?

The development of deep-sea drilling projects JOIDES and IPOD showed that some subduction zones did not have accretion prisms²¹⁻²³ and that generally their tectonic regime was of extension type. Using all types of geophysical and geological information, Uyeda and Kanamori²⁴ have classified the subduction zones into two types: Chile and Mariana type. For the latter, an extension occurs behind the subduction zone and then sediment injection is a possible mechanism. This process seems the most likely when considering a dynamic process for the subsidence instead of a forced injection of the subducting plate. This injection is not uniform, as shown by the JOIDES drills; some subduction zones, because of their strong distension, allow a fast injection (Central America, Marianas) whereas others with weaker distension produce accretion prisms (Caribbean, Java)^{24,25}. The variable intensity of the subduction processes and the distribution of subduction zone may create important geographical heterogeneities in the mantle.

Part of these sediments contaminates the arc volcanism as demonstrated by Kay²⁶, but an important part sinks into the mantle. When considering a 500–1,000-m-thick layer of pelagic sediments of average density 2.5, the same spreading characteristics used for ridges, a content of 30% carbonates and 1.5% organic carbon, we get $2.8\text{--}5.6 \times 10^{14} \text{ g}$ of injected carbon. This corresponds to the value already obtained for degassing, by a completely independent calculation.

Therefore, we may conclude that in the present conditions, the exogenous–endogenous cycle in the mantle is a steady state. Using the mean value of $4 \times 10^{14} \text{ g}$ for the exchange flux in both directions the lifetime of carbon in the exogenous cycle is $\tau_{\text{ex}} = 2 \times 10^8 \text{ yr}$. Let us now calculate the residence time in the

endogenous cycle. Considering that the convection system related to the plate motion only affects the upper mantle, as shown by isotopic and fluid mechanics studies, the mass of mantle material involved is $1 \times 10^{27} \text{ g}$. The carbon content of peridotites, for which $\delta^{13}\text{C}$ ranges from -12 to -7 (that is, primitive values), averages 400 p.p.m. (ref. 27). This corresponds to a carbon content of $4 \times 10^{23} \text{ g}$ for the upper mantle. The residence time of carbon in the upper mantle is thus $\tau = 1 \times 10^9 \text{ yr}$, five times longer than in the exogenous cycle.

These very short residence times are useful for explaining the present steady state. When calculating the residence time of the oceanic lithosphere in the mantle, we get $\tau = 3.7 \times 10^9 \text{ yr}$ (ref. 7). Thus, the residence time of carbon in the mantle is shorter and we must admit a much greater mobility for this element. This can be explained either by the fact that carbon is in the form of fluid (CO_2) for which the rate of transfer is large relative to the diffusion coefficient in solids, or in the form of atomic carbon, in defects, which Freund²⁸ has shown to be very mobile.

The mass injection of carbonates also explains the presence of CO_2 in the mantle, and the evidence²⁹ for CO_2 has been used elsewhere³⁰⁻³³ in petrological considerations. If the geodynamical behaviour of water in the mantle is similar to that of CO_2 and particularly its residence time, we may deduce important geochemical consequences: the $\text{H}_2\text{O}/\text{CO}_2$ ratio in the upper mantle could be equal to the chemical ratio during injection. The carbon and water contents of injected sediments average 4.5 and 3% respectively, so that the contents in water and carbon introduced through hydrothermal circulation in the

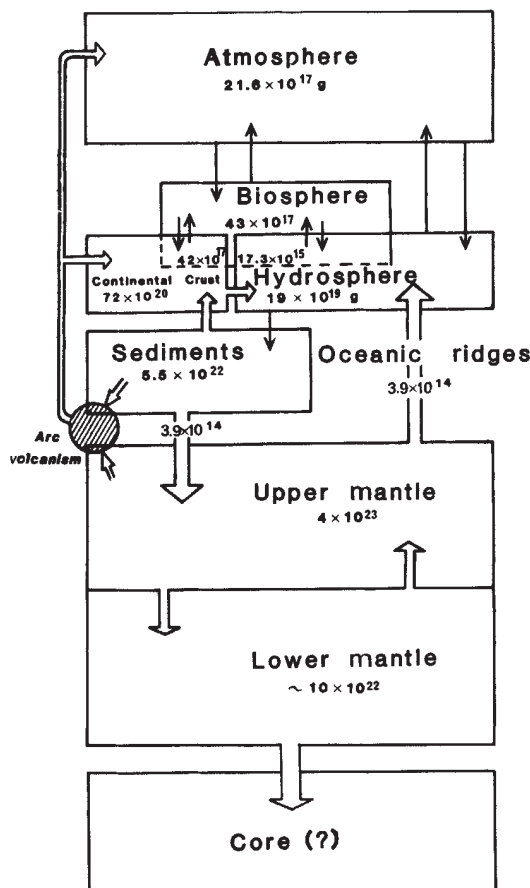


Fig. 2 Box model showing the different reservoirs for carbon and their mutual relationship. As our main interest is the exchange between exogenic and endogenous cycles, we have simplified the exogenic cycle. The masses of carbon in the reservoirs are expressed in grammes, those for the flux of exchange are in grammes per yr.

oceanic crust are 2 and 0.2% respectively. Considering a 2-km thick basaltic layer, we get 8.6×10^{14} g of water and a $\text{H}_2\text{O}/\text{CO}_2$ ratio in the mantle of 1.8.

We have neglected an important gas loss at volcanic arc areas—an acceptable approximation if considering a steady state. However, in future we will have to take this arc volcanism into consideration. In the steady-state hypothesis, we have the following equation for flux

$$F_{\text{ridge}} + F_{\text{arc}} = F_{\text{sediment}}$$

but the error on F_{sediment} and F_{ridge} is much too large and we cannot directly measure F_{arc} . No account has been taken for the moment of possible but unknown exchange between upper and lower mantle. Figure 2 shows the relationship proposed between these various reservoirs.

We have demonstrated that the mantle is degassing large amounts of CO_2 and H_2O . The study of isotopic composition of rare gases such as xenon and argon showed that the Earth has been suddenly degassed for ~95% in its first 50 Myr (ref. 6). If, as generally assumed, the carbon were present in the form of CO_2 and CH_4 in the atmosphere one may consider that carbon has been expelled from the mantle during this period. Thus, the exogenous reservoir of carbon was five times larger at this time than it is at present, and consequently the CO_2 content of the upper mantle much lower.

The sediment recycling began re-equilibrating both reservoirs as soon as carbonates formed in the primitive ocean and then the content of carbon in the mantle tends to increase when the content of CO_2 in the exogenic cycle decreases. At present, it is difficult to quantify such an evolution.

We point out the different history for the various mantle gases. For some of them, which are not carried heavily by sediments or subducted slabs, like probably Xe or Kr, their origins are residual after mantle outgassing. For some others, like CO_2 , they are completely recycled. In this respect it would be interesting to study the behaviour of N_2 or Cl for which recent studies seem to indicate an interesting recycling history. This comparative volatile geochemistry would enable us to reconstruct the history of the atmosphere, and also of sediment recycling and mantle history.

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1. Armstrong, R. L. *Rev. Geophys.* **6**, 175–199 (1968).
2. Allegre, C. J. *Tectonophysics* **81**, 109–132 (1982).
3. Allegre, C. J., Brevard, O., Dupré, B. & Minster, J. F. *Phil. Trans. R. Soc. A* **297**, 447–477 (1980).
4. O'Nions, R. K., Evensen, N. M. & Hamilton, P. J. *Phil. Trans. R. Soc. A* **297**, 479–493 (1980).
5. Magaritz, H. & Taylor, H. P. *Geochim. cosmochim. Acta* **40**, 215 (1976).
6. Staudacher, T. & Allegre, C. J. *Earth planet. Sci. Lett.* (in the press).
7. Armstrong, R. *Rev. Geophys.* **6**, 175–199 (1968).
8. Kurz, M., Jenkins, W. & Hart, S. R. *Earth planet. Sci. Lett.* (in the press).
9. Taylor, H. P. Jr, Frechen, J. & Degens, E. T. *Geochim. cosmochim. Acta* **31**, 407–430 (1967).
10. Deines, P. & Gold, D. P. *Geochim. cosmochim. Acta* **37**, 1709–1733 (1973).
11. Deines, P. *Geochim. cosmochim. Acta* **44**, 943–961 (1980).
12. Craig, H. *Geochim. cosmochim. Acta* **3**, 53 (1953).
13. Hoefs, J. *Contr. Miner. Petrol.* **41**, 277 (1973).
14. Pineau, F., Javoy, M. & Bottinga, Y. *Earth planet. Sci. Lett.* **29**, 413–421 (1970).
15. Pineau, F. thesis Univ. Paris 7 (1977).
16. Bottinga, Y. *J. phys. Chem.* **72**, 800 (1968).
17. Javoy, M., Pineau, F. & Iyama, I. *Contr. Miner. Petrol.* **67**, 35–39 (1978).
18. Pineau, F. & Javoy, M. *Earth planet. Sci. Lett.* (in the press).
19. Garrels, R. & MacKenzie, F. T. *Evolution of Sedimentary Rocks* (Norton, New York, 1971).
20. Hoefs, J. *Geochim. cosmochim. Acta* **29**, 399–428 (1965).
21. *Init. Rep. IPOD Drilling Proj.* **56–57 I** (1980).
22. *Init. Core Description Leg 60* (IPOD, Paris, 1978).
23. *Init. Core Description Leg 66* (IPOD, Paris, 1979).
24. Uyeda, S. & Kanamori, H. *J. geophys. Res.* **133**, 1049–1061 (1979).
25. Karig, D. E. *J. geophys. Res.* **76**, 2542–2561 (1971).
26. Kay, R. W. *J. Geol.* **68**, 497–522 (1980).
27. Garrels, R., MacKenzie, F. & Hunt, C. *Chemical Cycles and the Global Environment* (Kaufman, Los Altos, 1973).
28. Freund, F., Kathrein, H. & Wengeler, H. *Geochim. cosmochim. Acta* **44**, 1319–1333 (1980).
29. Roedder, E. *Am. Miner.* **50**, 1746–1782 (1965).
30. Eggle, D. H. in *Physics and Chemistry of the Earth* Vol. 9 (Pergamon, Oxford, 1975).
31. Brey, G. P. & Green, D. H. *Contr. Miner. Petrol.* **55**, 217–230 (1976).
32. Wyllie, P. J. & Huang, W. L. *Contr. Miner. Petrol.* **54**, 79–107 (1976).
33. Mysen, B. O. *Rev. Geophys. Space Phys.* **15**, 351–361 (1977).
34. Allard, P. & Javoy, M. (in preparation).
35. Delorme, H., Javoy, M., Cheminée, J. L. & Pineau, F. *Terra Cognita* Special Issue, 79 (1981).

Reversed magnetic polarity at an early Lower Palaeolithic site in Central Italy

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Isernia La Pineta central Italy is an extensive open-air archaeological site of early Lower Palaeolithic aspect. Radiometric and palaeomagnetic dating place it in the Matuyama reversed epoch, hence before 0.73 Myr. Its early date, stratified context, abundant stone tool industry of choppers and flakes and rich faunal remains make it one of the most important localities for the question of the earliest human colonization of Europe. The archaeological horizons (Fig. 1) were discovered during recent road-building operations at 4 m below surface near the city of Isernia (Upper Volturno Basin, Province of Molise; see Fig. 2). Excavations in 1979 and 1980 carried out in two areas (sectors I and II; Fig. 2) revealed dense scatters of limestone choppers, flint flakes and disarticulated bones of large mammals. Similar occurrences have been found elsewhere in the valley. Previous accounts^{1,2} are revised herein.

The archaeological levels are contained in fluvial and fluviolacustrine sediments pertaining to the principal fill of the Isernia Basin (Fig. 3)³. A lacustrine facies, evident at the base of this unit, ends in the north-east of the basin with banks of travertine often several metres thick. The principal fill covers depressions in a Pliocene erosional surface which retained some gradient as late as the early Pleistocene. Tectonic movements

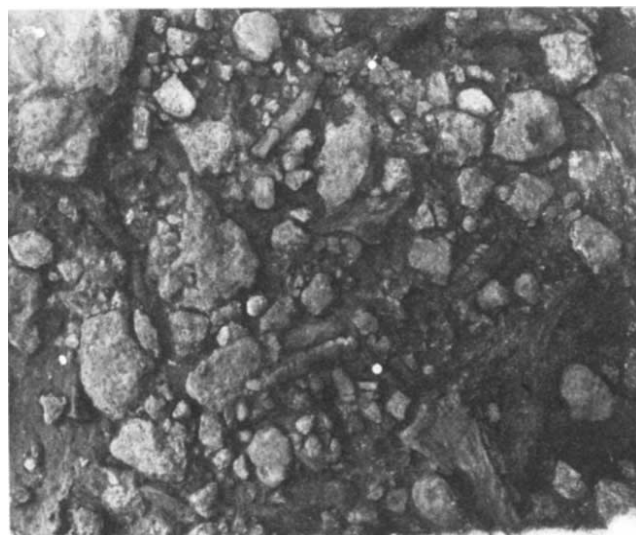


Fig. 1 Isernia La Pineta. Portion of archaeological horizon, sector I.

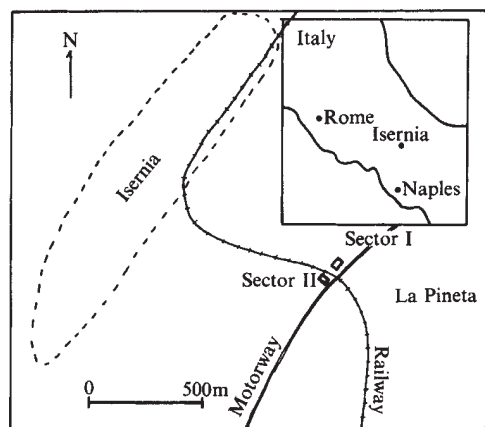


Fig. 2 Study area location map.

connected with volcanism disrupted this surface in the middle Pleistocene, causing a considerable increase in gradient and the deposition of coarse fluvial sediments.

Numerous beds of tuff are intercalated within fluvial layers of the principal fill⁴. Grey tufts, poor in pumice and sanidine, are found even in the lowermost lacustrine series. At higher elevations in the series tufts are mostly brown in colour; they are partly redeposited, but all are rich in sanidine and contain crystals of biotite and pumice fragments up to 1 cm in diameter. It is thought that the volcanic activity responsible for the deposition of these tufts took place at the same time as that of the nearby Rocca Monfina system.

Important neotectonic movements developed in the Isernia Basin, following Appenninic and anti-Appenninic directrices. Their most obvious effects consist of impressive stream capture and fault movement, involving surface rocks and/or Pleistocene deposits.

The Palaeolithic levels under consideration are sandwiched between the latest episodes of lacustrine sedimentation and the earliest fluvial deposits. The lower of the two archaeological layers encountered in sector I rests directly on a bed of travertine which shows signs of pedogenesis at its upper surface. A mantle of fluviatile muds, on which the upper occupation surface lies, covers the travertine. The upper archaeological surface was buried by sediments directly tied to an emission of tuff. The physical condition of the volcanic minerals, mainly pyroxenes and sanidines, excludes the possibility of significant fluvial transport. Fluvial sands and gravels were subsequently deposited, intercalated with tufts and palaeosols.

From the geostratigraphical standpoint the archaeological levels seem to represent brief events, whereas the sedimentary facies in which they are included were deposited over a longer time interval.

Recent studies suggest that the Rocca Monfina volcano, whose activity had a strong influence on the Isernia basin, was active from ~1.54 to 0.34 Myr (ref. 5). K-Ar dates obtained by the Amsterdam laboratory⁶ indicate that the first major phase of tuff deposition in the Isernia/Upper Volturno basin took place ~0.87–0.84 Myr.

A particularly distinctive feature of the latest depositional phase of the Isernia basin principal fill was a blanket of yellow tuff, found throughout the basin⁴. Two samples of sanidines from the yellow tuff in the Isernia La Pineta series, dated by the University of Rome laboratory, gave ages of 0.55 ± 0.05 and 0.47 ± 0.05 Myr (Fig. 3B), not significantly different.

A sample of the sediments immediately overlying the upper archaeological level in sector 1 gave an age of 0.73 ± 0.04 Myr. This date can be regarded as dating the archaeological level itself, as these sediments, directly tied to volcanic activity, buried the archaeological surface in a very short time. In view of the indications that the fluvial material from the top of the archaeological horizon up to tuff II was deposited rapidly, this

age is also in stratigraphical agreement with the date of 0.73 ± 0.07 Myr obtained by the Amsterdam laboratory. The sample (biotite) that gave the latter date was collected from a tuff in the Belfiore series (Fig. 3B) ~1 km north of the Isernia La Pineta section. The tuff is stratigraphically equivalent to tuff II at the latter section⁶.

Palaeomagnetic dating was done at the University of Pittsburgh. Oriented samples were measured in a cryogenic magnetometer. Polarities range from 'clearly reversed' to 'possibly reversed' and 'indeterminate' (because of heavy present field overprinting). No sample showed unambiguous normal polarity, so it is probable that deposition of the sediments sampled, and thus the archaeological layers as well, date to the Matuyama reversed epoch. There are no minor reversed episodes or excursions within the earlier half of the Brunhes to which the sample polarities could alternatively be ascribed. An attempt is being made to locate the Matuyama-Brunhes interface in the series.

The age indicated by palaeomagnetism and K-Ar (late early Pleistocene) is older than the age customarily ascribed to the Isernia fauna, and leads to doubts regarding the age of that faunal assemblage.

The archaeological layers have yielded a large sample of faunal remains, still in process of study. The materials from sector I, which are the better preserved, are being restored *in situ* and studied in detail as conservation is completed and they are removed.

The material from sector II consists of skeletal remains of large mammals, principally bison, bear, elephant, rhinoceros and hippopotamus. The parts represented, all fragmentary and mostly incomplete, include vertebrae, ribs, scapulae, pelvis, crania, and epiphyses of long bones. The only complete parts are teeth, second and third phalanges, carpals and tarsals—all parts that lack marrow.

The faunal material from sector I, mostly complete bones, is represented predominantly by crania of bison and a significant number of cranial and dental remains of the other large mammals. The long bones are mainly of elephants.

The large number of bones represent a small number of mammalian species. Most frequent is bison (*Bison* cf. *schoetensacki* Freudenberg), followed by rhinoceros (*Dicerorhinus* cf. *hemitoechus* Falconer), elephant (*Elephas antiquus* Falconer), and bear (*Ursus deningeri* von Reichenau); *Hippopotamus* sp. cf. *amphibius* L., *Sus scrofa* and *Hemionax* sp. are rare. Cervids are represented by possibly a single *Dama* individual.

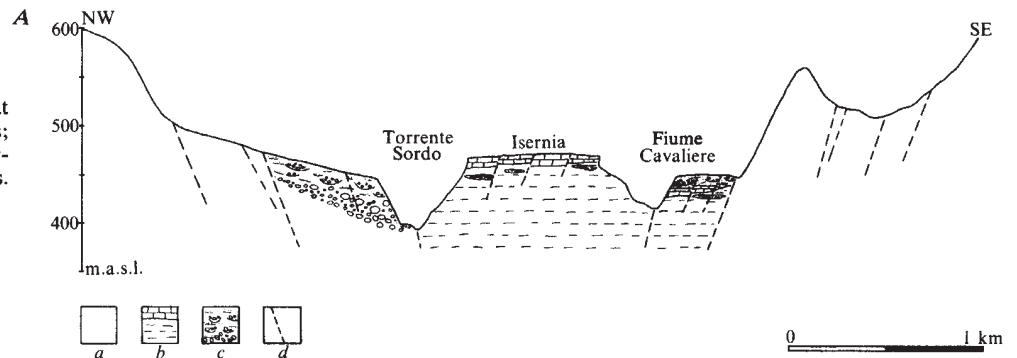
A few rodent bones were recovered from water-sifted fine sediments in the fauna-bearing layers. Because of scarcity and poor preservation, only a few isolated teeth could be identified (Fig. 4). The species represented are *Clethrionomys* sp., *Microtus arvalinus* Hinton, *Pitymys* cf. *arvaloides* Hinton, *Arvicola* cf. *mosbachensis* Schmindtgen, *Pliomys episcopalis* Meheley, and *Pliomys lenki* Heller.

The megafaunal assemblage has hitherto been ascribed to the middle Pleistocene. The micromammals, however, although scanty, confirm the indications from palaeomagnetic and radiometric dating that human activities at the site took place in the late early Pleistocene.

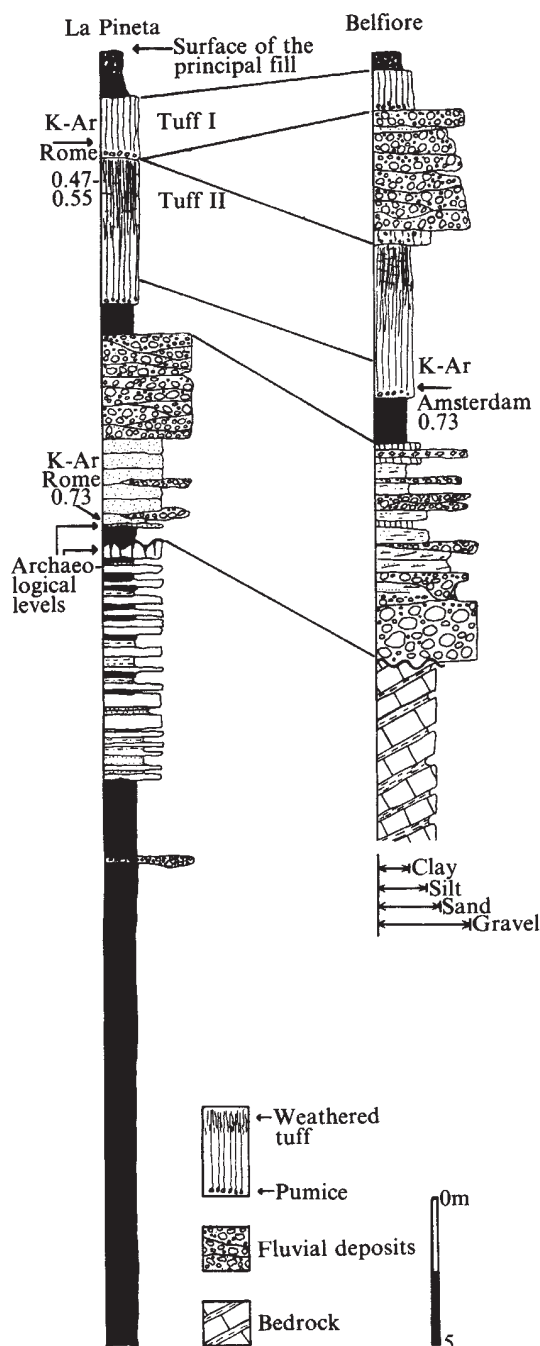
The low frequency of cervids—ordinarily the commonest large mammals encountered in middle Pleistocene faunas of the Mediterranean basin—calls for an attempt at explanation. The situation might be attributable to selection on the part of the hunters for animals with a greater meat yield, but the picture obtained from study of other Pleistocene sites is one in which hominids hunt all ungulates present in a region, with the result that the fauna reflects, if imperfectly, the real faunal population. Only carnivores are normally under-represented in human cultural deposits. Bears are an exception, but even their proportion is considerably lower than that of ungulates and other animals.

The tentative conclusion is that a specific climatic type with two seasons is represented at Isernia La Pineta: a brief wet

Fig. 3 A, geological profile at Isernia. a, Miocene–Eocene rocks; b, lacustrine sediments and travertines; c, fluvial sediments; d, faults. B, stratigraphy at Isernia.



B



season and a much longer arid season, resulting in an open steppe or grassland-steppe environment, well suited to a variety of pachyderm and bison species.

A continental molluscan fauna was studied from a clayey layer ~12 m below the upper archaeological horizon⁷. The species found are *Lymnaea truncatula*, *Vertigo pygmaea*, *Vertigo moulinsiana*, *Pupilla muscorum*, *Vallonia pulchella* and *Succinea oblonga*. Molluscan associations of this kind characterize both middle and late early Pleistocene cold phases of Central Europe.

In sector II, an archaeological horizon of ~70 m² was excavated. It had undergone weathering, which advanced the partial decalcification of the bones. Areas were encountered with heavy concentrations of lithic artefacts, most of small size. Tools include denticulates, notches, 'Tayacian'-like points (including some with dihedral ventral surfaces) and rare scrapers. There was a near absence of choppers. The faunal remains associated with the lithic scatters include a few fragments of diaphyses, mostly of small size and poorly preserved, and dental remains of bison, rhinoceros, elephant and bear.

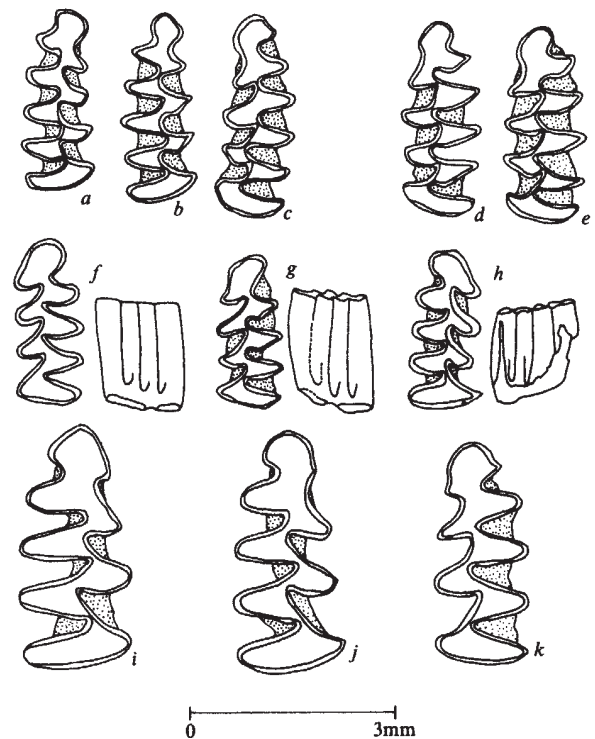


Fig. 4 Isernia La Pineta. First lower molars of micromammals (magnification $\times 10$). a–c, *Pitymys* cf. *arvaloides*; d–e, *Microtus arvalinus*; f–h, *Clethrionomys* sp.; i–k, *Arvicola* cf. *mosbachensis*.

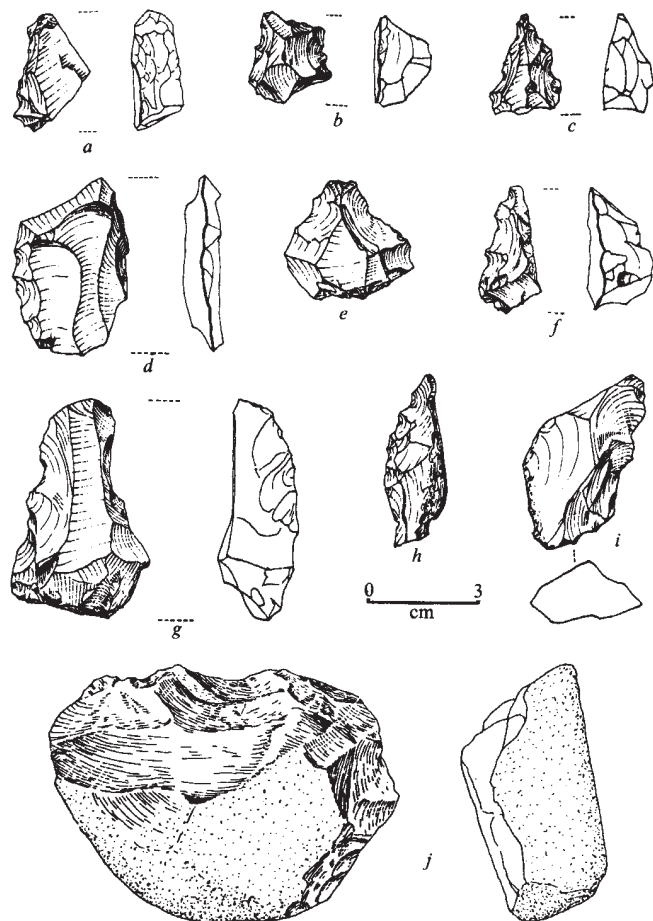


Fig. 5 Isernia La Pineta. Artefacts: a-i, denticulate tools on flint flakes; j, limestone chopper.

About 53 m² have been exposed in sector I. Several large blocks of limestone and travertine lay on the archaeological surface, which was covered with the bones of large mammals, with stone tools scattered among them. The disposition of the stone blocks and faunal remains strongly suggests human intervention. The faunal parts present are highly non-random. There were some 20 crania of bison; crania, mandibles and teeth of rhinoceros, hippopotamus and bear; tusks, molars and long bones of elephants. The stone tools—numbering in the thousands—can be divided into two major categories (Fig. 5). Choppers, nearly all of limestone river pebbles or cobbles, were highest in frequency. The other category comprises flake tools of flint, all much smaller than the choppers. Some can be identified as denticulates; there were only a few scrapers. Many were utilized but unmodified.

In 1980, an earlier cultural horizon was encountered in sector I beneath the one just described, separated from it by ~50 cm of culturally sterile deposits. This earlier layer, considerably lower in density of cultural materials than the one overlying it, rested on a bank of travertine. The lithic industry and faunal remains were in all respects similar to those from the upper layer.

It is not easy to find comparable sites for discussion. The few that have been ascribed to a similar age either yielded poor or ambiguous artefact inventories or do not clearly indicate a human presence. Others are surface scatters or lack secure provenience in some other way. Some of them are discussed elsewhere^{8,9}. The more secure of them at least support the existence of a non-Acheulian industry in the late early Pleistocene.

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planimetry and stereophoto coverage of the archaeological surface. Palaeomagnetic dating was aided by financial assistance from the National Geographic Society. Authors' specialities: M.Co., geomorphology; M.Cr., pedology and stratigraphy; M.C.D.M.F. and M.N., radiometric dating; D.E., malacofauna; A.McP., palaeomagnetism; R.V.O. and J.S., pedology and stratigraphy of the Volturno Basin; C.P., archaeology; B.S., palaeontology, biostratigraphy; V.S., palaeomagnetism.

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- Coltorti, M. *et al.* *Acts 10th Congress Un. int. Sci. Préhistoriques Protohistoriques*, Mexico City, 58-63 (1982).
- Coltorti, M., Cremaschi, M., Guerreschi, A., Peretto, C. & Sala, B. *Atti della XXIII Riunione Scientifica dell'Istituto Italiano di Preistoria e Protostoria*, Florence, 577-587 (1982).
- Coltorti, M. & Cremaschi, M. *Contributi Preliminari alla Carta neotettonica d'Italia* (Progetto Finalizzata Geodinamica. C.N.R., Rome, in the press).
- Sevink, J. & van Otterloo, R. *Geogr. Fis. Dinam. Quat.* (in the press).
- Giannetti, B., Nicoletti, M., & Petrucci, C. *Rc. Soc. Ital. miner. petrol.* **35**, 349-354 (1979).
- Sevink, J., Hebada, E. H., Priem, H. N. A., & Verschure, R. H. *J. archaeol. Sci.* **8**, 105-106 (1981).
- Esu, D. *Boll. Soc. geol. Ital.* **100**, 93-98 (1981).
- Radmilli, A. M. *Acts 9th Congr. Un. int. Sci. Préhistoriques Protohistoriques*, Nice, 35-74 (1976).
- Bordes, F. & Thibault, C. *Quat. Res.* **8**, 115-127 (1977).

A ziphodont mesosuchian crocodile from the Eocene of Algeria and its implications for vertebrate dispersal

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Fossils from a newly discovered, probably middle Eocene locality in southern Algeria provide the first evidence of an African terrestrial mesosuchian crocodilian with a ziphodont dentition (that is, with compressed, serrated teeth). Ziphodont mesosuchians were previously known mainly from South America and Europe, but have not been reported from North America. Several other groups of land vertebrates show a disjunct distribution in the Palaeogene, with representatives in Europe and South America, but no certain record in North America, where they should occur if, as has been suggested, dispersal had taken place from South America to Europe across North America. The discovery of a ziphodont mesosuchian in Algeria is the first tangible evidence that may support the alternative hypothesis of faunal connections between South America and Europe via Africa.

The crocodilian material described here was collected from the newly discovered El Kohol locality, near Brezina in southwestern Algeria, which is being excavated by a team from the universities of Paris and Oran under the direction of J. J. Jaeger. The continental vertebrate fauna, which is still being studied, includes a lungfish, a crocodilian, and various mammals (hyracoids and a form related to *Barytherium*). It seems to indicate an age older than the lower levels of the Fayum, possibly middle Eocene.

The crocodilians are represented at El Kohol by lower jaw fragments, isolated teeth, vertebrae and a fibula. The vertebrae are amphicoelous, which is indicative of the archaic suborder Mesosuchia. The fibula is elongated and slender, which may be related to a mainly terrestrial way of life. The teeth are especially interesting. They are definitely compressed laterally (although not as much as in some other ziphodont crocodilians) and bear two sharp, finely serrated carinae; the more posterior ones are rather blunt, with a finely wrinkled enamel, while the anterior ones are more pointed, with less distinct wrinkles. Two lower jaw fragments are available: one is an almost complete

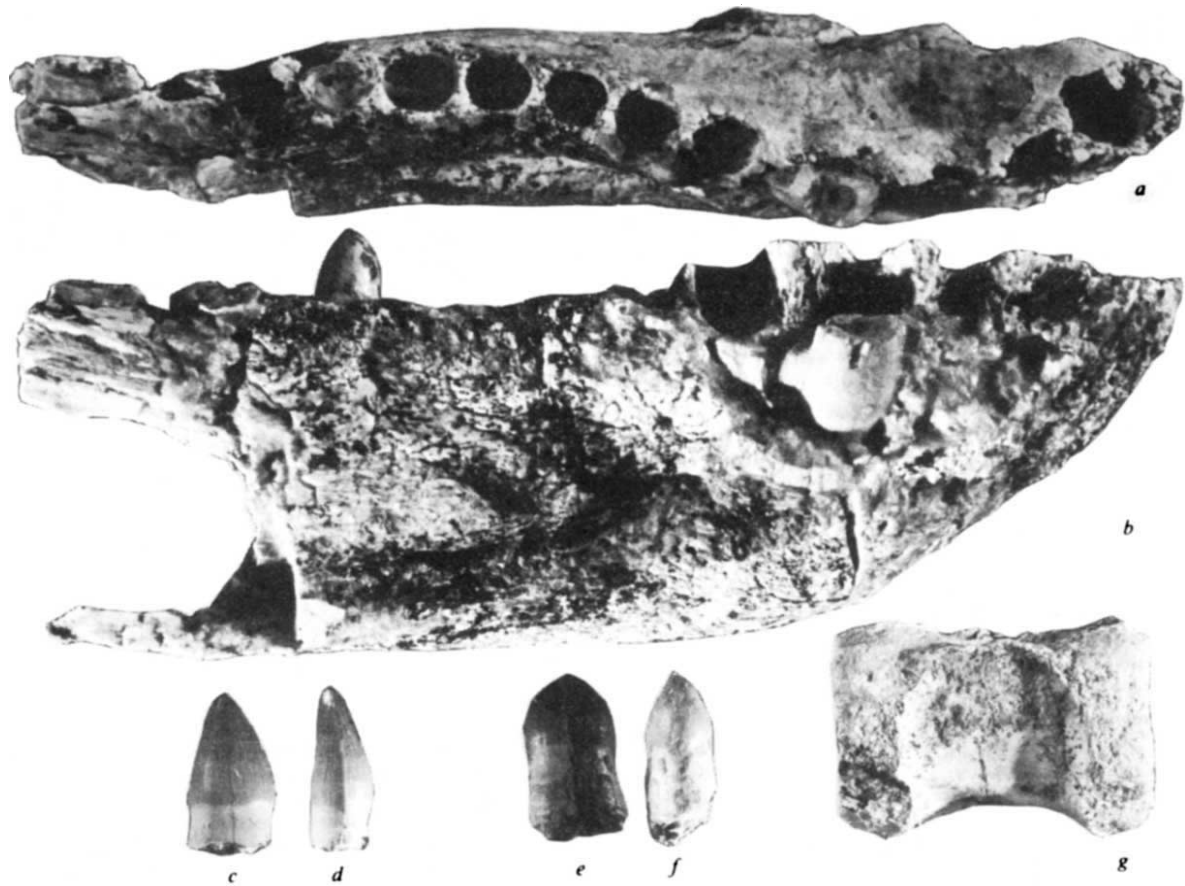


Fig. 1 Remains of a ziphodont mesosuchian crocodile from the Eocene of El Kohol, Algeria. *a, b*, Right dentary in dorsal (*a*) and lateral (*b*) views; *c, d*, two views of an isolated anterior tooth, showing lateral compression; *e, f*, two views of a posterior tooth, showing a blunt apex; *g*, lateral view of an amphicoelous caudal vertebra. $\times 1$. (Photographs by C. Abrial.)

right dentary, still bearing one complete tooth similar to the isolated ones described above, the other is the back part of a left mandibular ramus with the articular region. The dentary is remarkably deep and laterally compressed; its lateral surface shows a marked concavity. It bears 12 alveoli arranged in a sinuous row; the fourth alveolus is the largest. The dentary symphysis reached the level of the fifth alveolus, and the splenials too apparently took part in the symphysis. The articular region of the other jaw fragment is remarkable mainly because the surangular takes part in the jaw articulation.

The affinities of this ziphodont mesosuchian, which undoubtedly represents a new taxon, are still difficult to determine with complete accuracy. The depth of the dentary is reminiscent of the sebecosuchian *Baurusuchus*, a ziphodont mesosuchian from the Upper Cretaceous of Brazil¹. The articular region of the mandible is similar to that of *Sebecus*, a Tertiary sebecosuchian from South America², but it also closely resembles that of *Trematochampsia*, a non-ziphodont mesosuchian from the Upper Cretaceous of Niger and Madagascar^{3,4}. Although they are compressed and more distinctly serrated, the teeth too are somewhat reminiscent of the Trematochampsidae. Comparison with the few known ziphodont mesosuchians from Europe is difficult. The lower jaw of the Algerian form is quite unlike that of *Bergisuchus dietrichbergi* from the middle Eocene of Germany⁵. In *Iberosuchus macrodon*⁶, from the Eocene of Portugal and Spain, only the upper jaw is known. It should also be mentioned that the vertebrae from El Kohol are similar to those of a still undescribed ziphodont mesosuchian from the late Eocene of La Livinière (Hérault, southern France).

The biogeographical interpretation of the crocodilian from El Kohol depends on the phylogenetic relationships between the ziphodont mesosuchians of South America, Africa and

Europe. Independent evolution of the European forms is rather unlikely, for lack of suitable local ancestors. On the basis of the available material, however, the hypothesis that ziphodont mesosuchians appeared independently in South America and in Africa cannot be completely dismissed. The alternative hypothesis is that all three geographical groups are closely related, or, in other words, share a common ziphodont ancestry. Although the details of the phylogenetic history of these crocodilians and their systematic expression have still to be worked out, this more parsimonious interpretation of the record does not seem to be definitely contradicted by anatomical or chronological data. Pending future discoveries, it can be accepted as a working hypothesis that the South American Sebecosuchia, the El Kohol crocodilian, and the ziphodont mesosuchians from the European Eocene are closely related forms and not the products of convergent evolution. If this were the case the origin of the European forms would be of great interest.

In recent years, an increasing number of land vertebrates with South American affinities (that is, belonging to groups otherwise known mainly from South America) have been found in the European Eocene, some of which are unknown in the Cretaceous and early Tertiary of North America. Besides the ziphodont mesosuchians, such groups with a 'South American-European' distribution include amphibians (Ceratophryinae)⁷, land birds (Phorusracidae)⁸, and anteaters (Myrmecophagidae)⁹. A possible explanation for this peculiar distribution is that these groups originated in South America, and then spread to North America by way of a Central American land connection in the late Cretaceous, finally to reach Europe via the North Atlantic lands^{6,7,10,11}. Other land vertebrates, such as several groups of snakes¹² and the didelphine marsupials¹³, are recorded from the Palaeogene of North America and

probably went through such a biogeographical history. However, the main problem about accepting a similar situation for the ziphodont mesosuchians, the Ceratophryinae, the Phorusracidae and the Myrmecophagidae is that those groups have not been reported from the rich and fairly well-known late Cretaceous and Palaeogene fossil localities of North America. It has recently been suggested by C. Mourer-Chauviré¹⁴ that these land vertebrates may have reached Europe from Africa, after differentiating in Gondwana, either before or after South America became separated from Africa (in the latter case, dispersal across the Atlantic may have taken place along the Walvis-Rio Grande rises, which seem to have been subaerial in the late Cretaceous⁷). Evidence for faunal exchanges between Africa and Laurasia in the late Cretaceous and Palaeogene is still scanty, but does exist¹⁴; the recently discovered continental vertebrate fauna from the Palaeocene of Morocco (which, incidentally, does not indicate affinities with South America) is one of the most important elements in this connection¹⁵. However, there was hitherto no fossil evidence for the presence of the 'South American' groups of the European Eocene on the African continent. The discovery of a ziphodont mesosuchian in the Eocene of Algeria now seems to provide the first evidence that some of these land vertebrates may indeed have reached Europe from Africa.

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1. Price, L. I. *Notas prelim. Estud. Div. Geol. Miner. Bras.* **25**, 1-8 (1945).
2. Colbert, E. H. *Bull. Am. Mus. nat. Hist.* **87**, 221-270 (1946).
3. Buffetaut, E. *Geobios* **9**, 143-198 (1976).
4. Buffetaut, E. & Taquet, P. *Bull. Soc. géol. Fr.* **21**, 183-188 (1979).
5. Berg, D. E. *Abh. hess. Landesamt. Bodenforsch.* **52**, 1-105 (1966).
6. Antunes, M. T. *Communique Serus geol. Port.* **59**, 285-330 (1975).
7. Rage, J. C. *Cretaceous Res.* **2**, 65-84 (1981).
8. Mourer-Chauviré, C. *Geobios* **14**, 5, 637-646 (1981).
9. Storch, G. *Senckenberg. leth.* **61**, 3/6, 247-289 (1981).
10. Buffetaut, E. *Annls Paléont. (Vertébrés)* **66**, 1-18 (1980).
11. Buffetaut, E. & Rage, J. C. *Geobios* **15**, 267-269 (1982).
12. Rage, J. C. *C. r. somm. Séanc. Soc. géol. Fr.* **6**, 281-285 (1978).
13. Crochet, J. Y. *C. r. heb. Séanc. Acad. Sci. Paris* **D288**, 1457-1460 (1979).
14. Mourer-Chauviré, C. *Geobios* **15**, 268-269 (1982).
15. Cappetta, H. *et al. Geobios*, **11**, 2, 257-263 (1978).

Competition between female relatives in a matrilocal mammal

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Evolutionary theory predicts that in sexual organisms the average parents should invest equally in their male and female progeny¹ except where related males compete with each other to mate, when selection may favour parents that produce a female-biased sex ratio²⁻⁴. It has recently been suggested that in species where daughters adopt home ranges overlapping those of their mothers while sons disperse, competition for resources between female siblings may select for mothers who produce male-biased sex ratios⁵. We show here that in a mammal living in matrilocal, non-territorial groups—the red deer (*Cervus elaphus*)—the presence of resident female relatives depresses the reproductive success of adult females, providing a possible explanation of the apparent bias in parental investment towards male offspring in this species⁶.

Red deer hinds on the Isle of Rhum (Scotland) occupy home ranges of around 2 km² but spend most of their time within core areas of less than half this size (Fig. 1). Sons disperse from their mother's core area between the ages of 2 and 4 yr while daughters typically adopt core areas that overlap those of their mothers and older sisters. As a consequence of variation in

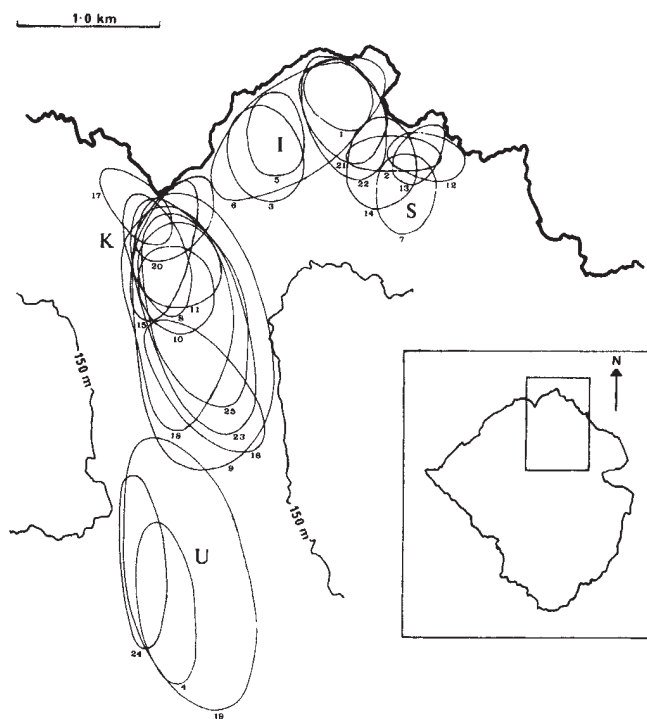


Fig. 1 Core areas of 25 different matrilocal groups of red deer hinds on the Isle of Rhum. Each ellipse shows the smallest area accounting for 65% of all sightings of members of that matrilocal group. The four divisions of the study area (U, K, I and S) are also shown. Numbers on ellipses identify individual groups and do not reflect the number of animals in them.

mortality, reproductive success and the proportion of male calves produced, the size of these matrilocal groups ranges over 2-12 breeding hinds. Related females associate with each other throughout their lives, though the core areas of different matrilocal groups overlap extensively and hinds commonly form parties consisting both of relatives and of members of other matrilocal groups.

Records of the reproduction of 43 individually recognizable hinds obtained between 1971 and 1979 showed that hinds belonging to matrilocal groups of above average size were less successful breeders than members of groups below average size (see Table 1a), an effect which occurred within all four divisions of our study area (Fig. 2). To check that the negative relationship between group size and reproductive success was not a consequence of any association between the size of matrilocal groups and the number of other deer using their range, we regressed our measures of the mean number of calves reared per year by individual hinds on the number of related hinds seen feeding in their core areas (*a*) and the number of unrelated hinds seen feeding in their core areas (*b*) over a 4-yr period. Reproductive success was only related to the first of these two values (*a*: $r = -0.343$, $t_{38} = 2.249$, $P < 0.05$; *b*: $r = 0.091$, $t_{38} = 0.503$, not significant (NS)). Moreover, the extent to which a group's size increased between the first and second halves of the study period was correlated with the extent to which the reproductive success of its members fell ($r_s = 0.360$, $t_{23} = 1.853$, $P < 0.05$, one-tailed).

The reduction in reproductive success among members of large groups was partly a result of an increase in the age at first breeding (only 47% of their members conceived for the first time as 2-yr-olds compared with 79% of members of small groups), partly of lower fecundity rates over the remainder of their lifespan and partly of increased calf mortality (see Table 1). The most likely cause of the reduction in the reproductive performance of females was increased competition for food between group members: the effects of population density on reproductive performance and survival are pronounced in this

Table 1 Results of studies showing competition between female relatives in red deer

	Large groups	Small groups	
<i>a</i> , Reproductive performance			
Median no. of calves surviving to 1 yr old reared per yr	0.500 (22)	0.675 (20)	$z = 2.594, P < 0.01$
Median no. of calves born per yr	0.695 (16)	0.847 (16)	$U = 71.5, P < 0.05$
% Male calves dying in first yr of life	36.4 (118)	16.2 (117)	$G = 12.60, \text{d.f.} = 1, P < 0.001$
% Female calves dying in first yr of life	22.1 (95)	24.1 (87)	NS
% Males dying as yearlings	22.7 (75)	6.5 (98)	$G = 10.19, \text{d.f.} = 1, P < 0.01$
% Females dying as yearlings	9.1 (75)	9.3 (66)	NS
Birth sex ratio	127:100 $n = 123$	140:100 $n = 142$	NS
<i>b</i> , Kidney fat index			
Calves	Males 1.5	Females 1.6	$n = 26, 24$
Yearlings	1.6	2.0	$n = 38, 36$
2 Yr olds	1.9	3.5	$n = 67, 51$

a, Reproductive performance of hinds belonging to large versus small matrilineal groups (all P values two-tailed). A large group was one above the median size for the relevant division of the study area (see Fig. 1), a small group one that was below this size (sample sizes are given in parentheses). *b*, Kidney fat indices (weight of kidneys and perinephric fat divided by weight of kidneys) for calves and yearlings shot in winter in central Scotland (from ref. 9).

population and the frequency of aggressive interactions between hinds was greatest in those parts of the study area where population density was highest⁷.

The size of a mother's group influenced the survival of her sons but had little or no effect on the survival of her daughters (Table 1*a*)—although both sons and daughters up to 1 yr old used the same food sources as their adult female relatives⁷. Feeding competition between male offspring and their female relatives was probably the principal cause of the increased mortality of young males in large groups as individuals that died had no more resident male relatives than those that survived (median test $G = 1.495, \text{d.f.} = 1, \text{NS}$). Reduced survival in conditions of food shortage in adolescent males compared with females of the same age is common among dimorphic mammals⁷⁻¹³. One contributory factor is probably the associated tendency for young males to lay down less body fat than females (see Table 1*b*). This sex difference may have evolved because the lifetime reproductive success of males is more strongly influenced by their adult body size than that of females⁷, adult size is closely related to early growth and selection may consequently favour the increased allocation of nutritional resources by young males to muscular and skeletal growth instead of to the insurance policy provided by fat reserves^{7,17}.

We previously⁶ demonstrated that in the same population hinds invested more heavily in their sons than their daughters

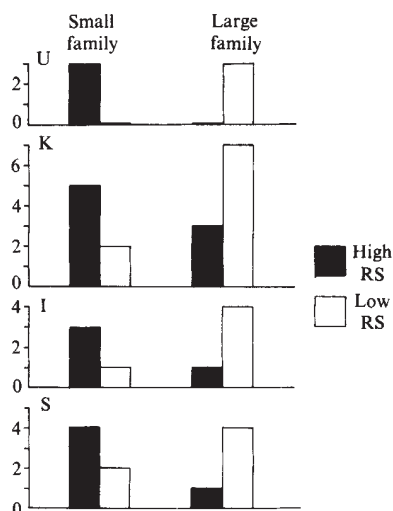


Fig. 2 Number of hinds belonging to large versus small matrilineal groups whose reproductive success (mean number of calves reared per year) are above versus below the average for the division of the study area where their home range was located (see Fig. 1). A large group was one above the median size for the relevant division of the study area, a small group one below this size.

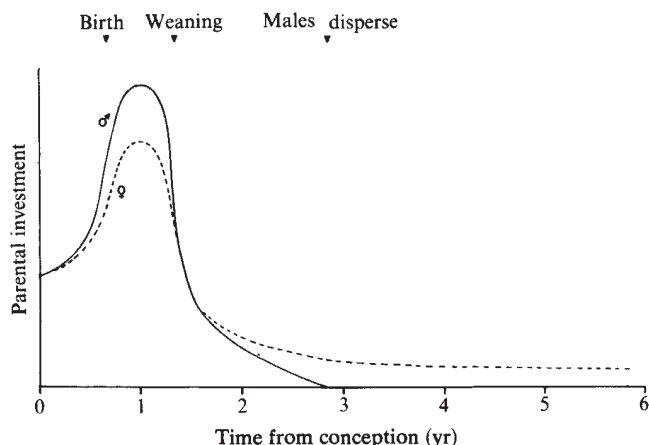


Fig. 3 Hypothetical plot of the changes in the distribution of parental investment¹⁴ by red deer hinds to their male and female calves.

before weaning: male calves sucked more frequently and mothers that had reared sons were less likely to calve the following year and, if they did so, calved later than mothers who had reared daughters. Despite the increased costs of producing male offspring and their higher mortality, the sex ratio of calves at weaning showed a consistent though non-significant bias towards males (118 male:100 female, $n = 347$) and we argued that our results were difficult to reconcile with Fisher's theory that parents should divide their total investment equally between their male and female progeny¹.

The results described here provide a possible resolution to this problem. Although sons may be more costly to rear than daughters, they disperse widely through the population and competition between adult male siblings either for food or for mates is uncommon. In contrast, daughters adopt home ranges overlapping those of their mothers and compete with each other as the size of the matrilineal unit grows.

The situation can, perhaps, be most easily viewed from the standpoint of the mother, who invests¹⁴ more heavily in her sons than her daughters before weaning but more heavily in her daughters than her sons afterwards (see Fig. 3). This need not imply that mothers treat their daughters differently from other conspecifics (although related hinds do, in fact, tolerate each other in close proximity while feeding^{7,15}) because in this context all costs of producing daughters should be considered as forms of investment. The temporal change in the bias of investment is likely to increase the mother's fitness, for our research indicates that the lifetime reproductive success of males is more strongly influenced by their body size and their early growth rates (see above) than by differences in the quality

of the home range they occupy as adults, while the reverse is true of females^{6,7}.

Competition among female relatives for access to local resources⁵ is likely to be widespread among mammals because males commonly disperse from their natal area while females remain in the locality where they are born¹⁶. A recent survey of the available literature shows that significantly male-biased fetal and birth sex ratios are substantially more common than female-biased ones in mammals¹⁷. However, a possible alternative explanation is that male-biased birth sex ratios have been favoured because they compensate for increased pre-weaning mortality among males¹ which may only occur in populations close to carrying capacity. Unfortunately, insufficient data are available to determine whether male-biased sex ratios are confined to species where daughters seldom disperse from their natal area, as the theory of local resource competition would predict.

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1. Fisher, R. A. *The Genetical Theory of Natural Selection* (Clarendon, Oxford, 1930).
2. Hamilton, W. D. *Science* **156**, 477-488 (1967).
3. Charnov, E. L., Hartogh, R. L., Jones, W. T. & van den Assem, J. *Nature* **289**, 27-33 (1981).
4. Taylor, P. D. *Nature* **291**, 64-66 (1981).
5. Clark, A. B. *Science* **201**, 163-165 (1978).
6. Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. *Nature* **289**, 487-489 (1981).
7. Clutton-Brock, T. H., Guinness, F. E. & Albon, S. D. *Red Deer: The Behavior and Ecology of Two Sexes* (Chicago University Press, 1982).
8. Glucksman, A. *Biol. Rev.* **49**, 423 (1974).
9. McClure, P. A. *Science* **211**, 1058-1060 (1980).
10. Robinette, W. L., Gashwiler, J. S., Low, J. B. & Jones, D. A. *J. Wildl. Mgmt* **21**, 1-16 (1957).
11. Widdowson, E. M. *Proc. Nutr. Soc.* **35**, 175-176 (1976).
12. Ralls, K., Brownell, R. C. & Ballou, J. *Rep. int. Whaling Commn* **2**, 233-243 (1980).
13. Grubb, P. in *Island Survivors: The Ecology of the Soay Sheep of St Kilda* (eds Jewell, P. A., Milner, C. & Boyd, J. M.) 242-272 (Athlone, London, 1974).
14. Trivers, R. L. in *Sexual Selection and the Descent of Man 1871-1971* (ed. Campbell, B.) 136-179 (Aldine-Atherton, Chicago, 1972).
15. Guinness, F. E., Hall, M. J. & Cockerill, R. A. *Anim. Behav.* **27**, 536-544 (1978).
16. Greenwood, P. J. *Anim. Behav.* **28**, 1140-1162 (1980).
17. Clutton-Brock, T. H. & Albon, S. D. in *Current Problems in Sociobiology* (ed. King's College Sociobiology Group) 223-247 (Cambridge University Press, 1982).
18. Pianka, E. R. *Evolutionary Ecology* (Harper & Row, New York, 1973).
19. Mitchell, B., Staines, B. W. & Welch, D. *Ecology of Red Deer: A Research Review Relevant to their Management in Scotland* (Institute of Terrestrial Ecology, Cambridge, 1977).

Structure of physiologically classified neurones in the kitten dorsal lateral geniculate nucleus

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The mammalian forebrain, including the dorsal lateral geniculate nucleus (LGN_d) and the visual cortex, continues both structural and functional development postnatally and is therefore a useful model for the study of developmental processes in the central nervous system (CNS). We report here the first description and comparison of the structural development of individual, functionally identified neurones in the mammalian forebrain. This comparison is made for the three main cell groups of the central visual pathways (W-, X- and Y-cells), in the neonate and the adult. In the adult, these three classes of neurones have different characteristic electrophysiological properties¹⁻⁹ and relay information in parallel about different

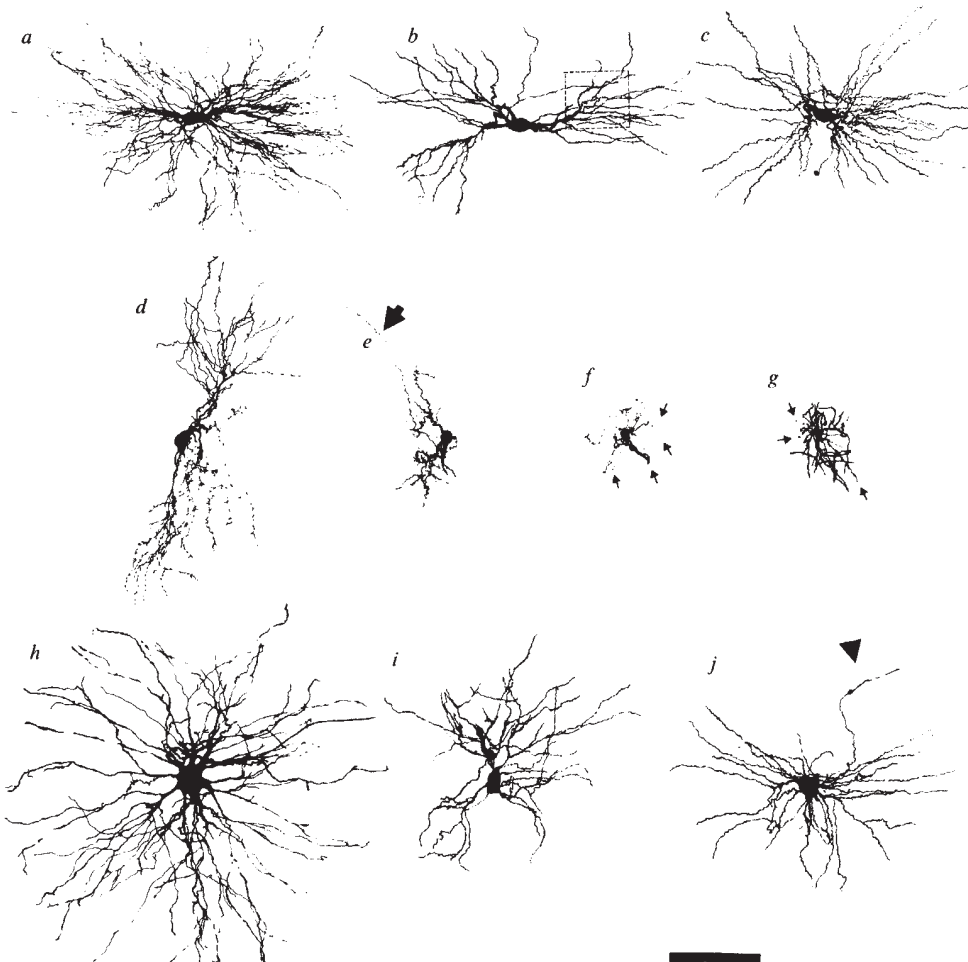
features of a visual scene^{8,9} from the retina through the LGN_d to the visual cortex. In addition, each of the three functional cell types has a characteristic structure in the adult^{10,11}. By injection of the enzyme marker substance, horseradish peroxidase, into electrophysiologically identified neurones, the present study demonstrates that each of these functional classes of neurones also has a characteristic morphology in the neonate (in the LGN_d of kitten 3-4 postnatal weeks of age). However, striking differences in the rates of maturation are seen. The W-cells are already mature at this age. The X-cells are the least developed. Surprisingly, some Y-cells are mature. Due to the susceptibility of Y-cells to an abnormal visual environment during development¹²⁻¹⁸, they had previously been thought to be slower to mature.

Kittens were anaesthetized with 1.5% halothane in a 1:1 mixture of N₂O:O₂ for all surgical procedures. All wound margins were infiltrated with 1.0% lidocaine. The kittens were maintained on a 70:30 mixture of N₂O:O₂ during the experiment. When an increase in heart rate occurred (above 175 beats per min) 1% halothane was administered for 5-10 min until heart rate returned to normal. Electrophysiological data were not taken during halothane administration. Respiratory control, P_{CO₂} regulation, temperature control, intravenous administration of paralytic solutions, refractions, placement of stimulating electrodes and preparation of recording micropipettes filled with horseradish peroxidase (HRP) have all been described previously¹⁰ for adult cats. The only difference for the kitten experiments was a reduced rate of administration and concentration of paralytic (12.0 mg per kg per h gallamine triethiodide and 0.5 mg per kg per h (+)tubocurarine at 2 ml per h).

Neurones in the LGN_d were initially characterized by extracellular recording. The following physiological parameters were determined for each cell: (1) the position and size of the receptive field, (2) the response type to a stationary stimulus (excitatory response to light onset or offset), (3) the regularity of the response to moving targets, (4) the strength of the receptive field surround, (5) the regularity of the response to repeated presentation of a stationary stimulus to the receptive field centre, (6) the linearity of spatial summation within the receptive field (using a sinusoidally modulated grating pattern on a cathode ray tube at an appropriately high spatial frequency^{4,5,8,10}), (7) ocular dominance, and (8) the latency of response to electrical stimulation of the optic chiasm. The cell was then penetrated with the micropipette, and the receptive field properties were rechecked while recording intracellularly¹⁰. HRP was then injected into the cell iontophoretically. Only one cell was injected in a single electrode penetration and no more than three cells were injected in an LGN_d. The kittens were then deeply anaesthetized with pentobarbital and perfused transcardially with a phosphate-buffered 2.5% glutaraldehyde: 1.0% paraformaldehyde fixative 2-10 h after the injections. Tissue preparation and reaction with 3-3'-diaminobenzidine (DAB) were as previously described¹⁰. In some cases, the Cobolt intensified DAB reaction¹⁹ was used.

Eighteen neurones in the 3-4-week-old kittens were characterized both physiologically and structurally (seven W-cells, seven X-like cells and four Y-cells). Of the seven W-cells (all located in the C-laminae of the LGN_d), all had morphological features similar to those of the adult. Five of these seven kitten W-cells were highly responsive to moving targets. They responded in a regular fashion to repeated visual stimuli, with little decline in the vigour of the response from trial to trial. (This early physiological maturation of movement sensitive neurones in kitten C-laminae was also observed by Daniels *et al.*²⁰.) This was in contrast to many X-like cells in the A-laminae of the kitten LGN_d. These X-like cells would cease responding vigorously to visual targets after one to five trials. Five of the seven W-cells responded to a sinusoidally counterphased grating of low spatial frequency (0.2-0.5 cycles per degree at a contrast of 0.6 and a modulation rate of 0.3 Hz). These were the highest spatial frequencies they would respond to. All five

Fig. 1 Drawings of W- (*a-c*), X- (*d-g*), and Y- (*h-j*) cells from the cat dorsal lateral geniculate nucleus (LGN_d). *a*, *d* and *h* are from adult cats. *b*, *c*, *e-g*, *i* and *j* are from 3-4-week-old kittens. All of the above cells were physiologically classified and then injected intracellularly with horseradish peroxidase. Drawings were made with a $\times 100$ oil objective (numerical aperture = 1.32) and $\times 10$ eyepieces with a drawing tube attachment. A Kodak Wratten 48A deep blue filter was used to enhance contrast when the 3-3' diaminobenzidine reaction was used to locate the HRP filled cells. *a-c*, W-cells: note the horizontal orientation of the dendritic trees of the typical adult (*a*) and the 3-4-week old kitten (*b* and *c*) W-cells. Note the very similar appearance of the adult and kitten W-cells. *d-g*, X-cells: note the vertical orientation of the dendritic tree of the typical adult (*d*) X-cell. These X-cells in the adult also confined their dendrites to the laminae of origin. The 3-4-week old kitten X-cells (*e-g*) were morphologically immature. The arrow in *e* indicates this cell's axon. This axon had collaterals both within the laminae of origin and above the laminae in the perigeniculate nucleus (PGN). The arrows in *f* and *g* indicate terminal swellings on the ends of the dendrites of two of the very immature looking X-like cells in the kitten. *h-j*, Y-cells: the adult Y-cells (*h*) had large somata, and thick radially oriented dendrites that crossed laminar boundaries. Their axons had collaterals that innervate the PGN. The 3-4-week old kitten (*i-j*) Y-cells had a similar appearance (only smaller). They too had radially oriented dendrites that cross laminar borders and innervate the PGN (arrow in *j*). Scale bar = 100 μm for all drawings and is drawn parallel to the laminar borders of the LGN_d.



of these kitten W-cells showed linear summation within their receptive fields. Two of these kitten W-cells are illustrated in Fig. 1*b*, *c*. Note the W-cell from an adult cat's LGN_d illustrated in Fig. 1*a* for comparison. The dendritic structure of the kitten W-cells is quite similar in orientation and detail to that of the adult. The dendrites are oriented parallel to the LGN_d lamination. In addition to having an adult-like dendritic structure, the somata of the kitten W-cells are large (Fig. 2). The sizes of the kitten W-cells' somata overlap only with the large adult W-cells (range = 210–360 μm^2 in kitten and 80–330 μm^2 in adult). In summary, 3–4 weeks after birth, physiologically identified W-cells are structurally mature, particularly those that are sensitive to moving visual stimuli.

On the other hand, the kitten X-like cells are immature at this age. The seven X-like cells all responded to the sinusoidally modulated grating pattern in a linear fashion^{4,5}. All were located in the A laminae of the LGN_d. In addition, they all were poorly responsive or not responsive at all to a large target of contrast opposite to their receptive field centre sign moved at moderate speeds (60–100 deg s^{-1}) through their receptive fields. Receptive field surround responses were very weak. No X-like cell responded in an excitatory fashion when the receptive field surround was presented with an annular stimulus of appropriate contrast. However, large stimuli activating both centres and surrounds simultaneously caused a reduced response in all seven X-like cells, indicating an active antagonistic surround mechanism. Latency of response to optic chiasm stimulation overlapped too much between X- and Y-cells in the 3–4-week old kittens to be a reliable correlator with other X- and Y-cell physiological properties. The structure of three of the most physiologically

mature X-like cells was like that illustrated in Fig. 1*e*. These X-like cells had receptive field centre sizes ranging over 0.8–1.6°. The cell illustrated in Fig. 1*e* had many complex appendages along its dendrites which were oriented orthogonal to the lamination of the LGN_d (Fig. 1*d* is an example of an adult X-cell for comparison). The other four X-like cells had morphological features that were quite different from any adult LGN_d cells. Two examples of these are illustrated in Fig. 1*f*, *g*. Note the small dendritic arborizations and lack of obvious vertical orientation. These cells have few appendages along their dendrites. However, the dendrites terminate in swellings (arrows) which may be growth appendages. The four X-like cells with this immature structure were also the most physiologically immature (rapid decrement of a vigorous response to a visual stimulation after only one or two trials with receptive field centre sizes of 2.1–3.5°). The distribution of soma sizes for the kitten X-like cells overlaps only with the lower end of the adult X-cell size range (Fig. 2, range = 80–182 μm^2 in kitten and 80–430 μm^2 in adult). In summary, the X-like cells in the 3–4-week old kitten LGN_d have some mature physiological properties (linear spatial summation and poor responsiveness to fast moving targets) and some immature physiological properties (large receptive field centres, very weak receptive field surrounds and rapid fatiguing of the cells' responses to repetitive stimulation). As a group, the X-like cells are the most anatomically immature cells in the LGN_d. Three of seven of these X-like cells had morphological features similar to adult X-cells except for smaller somata and dendritic spread (Fig. 1*e*), while the other four X-like cells had very short dendrites with few typical X-cell dendritic appendages. No X-cells in the

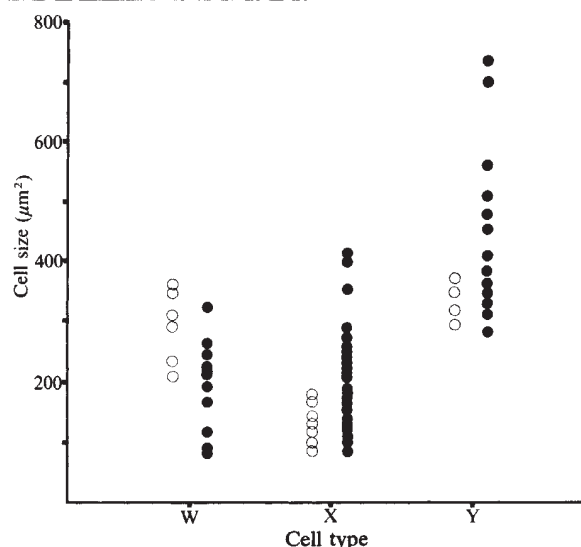


Fig. 2 Distribution of soma sizes for W-, X- and Y-cells in the 3-4-week old kitten LGN_d and the adult cat LGN_d. Open circles, kitten cell sizes; filled circles, adult cell sizes. Adult cell sizes are taken from study of Friedlander *et al.*¹⁰. All cells were physiologically classified and injected intracellularly with HRP. Tissue was vibratomed, reacted with DAB, dehydrated in alcohols and cleared in xylenes in an identical fashion in the kittens and adults. Soma sizes were measured with an electronic planimeter (10 measurements averaged for each cell). Note that the kitten W-cells are as large as the largest adult W-cells, while the kitten X- and Y-cell sizes only overlap with the lower end of the adult range.

kitten or the adult had dendrites which crossed laminar boundaries in the LGN_d.

The four kitten Y-cells all responded in a nonlinear fashion to the sinusoidal grating modulation. They all responded regularly to a large visual target moved rapidly through the receptive fields (both of these are features of adult Y-cells¹⁰). Repeated presentation of stationary visual stimuli elicited consistent responses from the Y-cells. This is in contrast to the X-like cells, which fatigued readily. The Y-cells responded to appropriate stimulation of the receptive field surround in an excitatory fashion. In addition, simultaneous activation of receptive field centres and surrounds antagonized the response. These properties are similar to adult Y-cells. All of the Y-cells were located in the A-laminae as were the X-like cells. Two of these kitten Y-cells are illustrated in Fig. 1*i, j*. Note the adult Y-cell in Fig. 1*h* for comparison. These physiologically mature Y-cells of the kitten are similar in morphology to their counterparts in the adult. However, note that the size of the somata and area covered by the dendrites is somewhat smaller than in the typical adult Y-cell. The dendrites of all four kitten Y-cells crossed laminar borders in the LGN_d as did the dendrites of 18 of 19 of the adult Y-cells. In addition, the axons of all four kitten Y-cells innervated the perigeniculate nucleus (PGN) as illustrated by the arrow in Fig. 1*j*. This type of axon collateral is quite common for adult Y-cells¹⁰. The somata of the kitten Y-cells that show this early physiological maturation are all in the lower end of the adult Y-cell size range (Fig. 2, range = 290–380 μm^2 in kitten and 285–750 μm^2 in adult). In summary, there is a population of Y-cells in the LGN_d of 3-4-week old kittens that has mature physiological properties (nonlinear spatial summation within their receptive fields, consistent responses to repeated presentations of stationary visual targets, and responsiveness to targets moved rapidly through their receptive fields). These cells are also structurally very similar to adult Y-cells (relatively large somata, thick radially arranged dendrites, dendrites that cross laminar boundaries and innervation of the perigeniculate nucleus).

Until recently little was known about the postnatal maturation of the structure of LGN_d A-laminae cells^{21,22} and nothing

of the development of the cells in the C-laminae. The data presented in this report support the hypothesis of differential morphological development of the functional subgroups of LGN_d neurones. A population of Y-cells matures by 3-4 postnatal weeks while X-like cells are later to mature. In previous electrophysiological studies of kitten LGN_d cells²⁰ and retinal ganglion cells^{23,24} a definitive property of adult Y-cells (non-linearity of spatial summation) was observed infrequently. This observation along with the reported greater sensitivity of the Y-system to the effects of monocular visual deprivation¹²⁻¹⁸ during development suggests that the Y-cells are late to mature. It was therefore surprising to find that as a group the Y-cells of the kitten were more structurally mature than the X-like cells. However, other Y-cells may still mature later. At least two possible mechanisms could explain this. Some Y-cells may be so immature as not to generate action potentials and would be missed by the micropipettes. Alternatively, some of the X-like cells in the kitten may develop into Y-cells later. The neural circuitry of the retina which contributes the nonlinear subunits⁵ may not be mature yet. An LGN_d Y-cell that is innervated by such a retinal Y-cell would respond to a modulated grating pattern in a linear fashion and would be physiologically classified as an X-cell in the kitten. Since the results of other physiological tests used such as latency to optic chiasm stimulation overlap in the kitten, such a cell can only be classified as X-like. The possibility that the LGN_d Y-cell population may be further subdivided into groups that mature at different rates is further supported by studies of monocular visual deprivation¹²⁻¹⁸. These studies have all found that some normally responsive Y-cells develop in the LGN_d laminae innervated by the visually deprived eye. However, fewer of these normal Y-cells occur in deprived laminae versus normal laminae. Other Y-cells develop abnormal structure and function after monocular visual deprivation¹⁷. The early maturing (structurally and functionally) Y-cells described in the present study may represent those normal Y-cells found in the adult that are resistant to the effects of visual deprivation.

The early morphological development of the W-cells in the C-laminae includes somatic growth (Fig. 2) as well as dendritic structure (Fig. 1*b, c*). This contrasts with the case in the A-laminae where somatic growth continues until 5-6 postnatal weeks of age²⁵⁻²⁷. If the soma size of the LGN_d neurones relates to the size of their axonal arborization in visual cortex^{28,29}, the W-cells may thus have a relatively larger sphere of influence in the young kitten's cortex than X- and Y-cells. Along with the early physiological maturity of movement sensitive W-cells, this would suggest that the W-pathway may have an important role in visual processing in the young animal.

The variable rates of physiological maturation of neonatal LGN_d cells are likely to depend on the development of the retinal ganglion cells that innervate them. However, the structural development of the LGN_d cells may depend on intrinsic factors as well as retinal input.

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- Enroth-Cugell, C. & Robson, R. G. *J. Physiol., Lond.* **187**, 517-552 (1966).
- Cleland, B. G., Dubin, M. W. & Levick, W. R. *J. Physiol., Lond.* **217**, 473-496 (1971).
- Hoffmann, K.-P., Stone, J. & Sherman, S. M. *J. Neurophysiol.* **35**, 518-531 (1972).
- Hochstein, S. & Shapley, R. M. *J. Physiol., Lond.* **262**, 237-264 (1976).
- Hochstein, S. & Shapley, R. M. *J. Physiol., Lond.* **262**, 265-284 (1976).
- Stevens, J. K. & Gerstein, G. L. *J. Neurophysiol.* **39**, 213-238 (1976).
- Wilson, P. D., Rowe, M. H. & Stone, J. *J. Neurophysiol.* **39**, 1193-1209 (1976).
- Lehmkuhle, S., Kratz, K. E., Mangel, S. C. & Sherman, S. M. *J. Neurophysiol.* **43**, 520-541 (1980).
- Sur, M. & Sherman, S. M. *J. Neurophysiol.* **47**, 869-884 (1982).
- Friedlander, M. J., Lin, C.-S., Stanford, L. R. & Sherman, S. M. *J. Neurophysiol.* **46**, 80-129 (1981).
- Stanford, L. R., Friedlander, M. J. & Sherman, S. M. *J. Neurosci.* **1**, 578-584 (1981).
- Sherman, S. M., Hoffman, K.-P. & Stone, J. *J. Neurophysiol.* **35**, 532-541 (1972).
- Kratz, K. E., Webb, S. V. & Sherman, S. M. *J. comp. Neurol.* **181**, 615-625 (1978).
- Mower, G. D., Burchfiel, J. L. & Duffy, F. H. *Devl Brain Res.* **1**, 418-424 (1981).

15. Zetlan, S. R., Spear, P. D. & Geisert, E. E. *Vision Res.* **21**, 1035–1039 (1981).
16. Friedlander, M. J. & Stanford, L. R. *Invest. Ophthalm. vis. Sci.* **22**(3), 236 (1982).
17. Friedlander, M. H., Stanford, L. R. & Sherman, S. M. *J. Neurosci.* **2**, 321–330 (1982).
18. Geisert, E. E., Spear, P. D., Zetlan, S. R. & Langsetmo, A. *J. Neurosci.* **2**, 577–588 (1982).
19. Adams, J. C. *Neuroscience* **2**, 141–145 (1977).
20. Daniels, J. N., Pettigrew, J. D. & Norman, J. L. *J. Neurophysiol.* **41**, 1394–1417 (1978).
21. Mason, C. A. *Soc. Neurosci. Abstr.* **7**, 675 (1981).
22. Friedlander, M. J. *Soc. Neurosci. Abstr.* **7**, 140 (1981).
23. Hamasaki, I. I. & Sutija, V. G. *Expl Brain Res.* **35**, 9–23 (1979).
24. Rusoff, A. C. & Dubin, M. W. *J. Neurophysiol.* **40**, 1188–1198 (1977).
25. Garey, L. J., Fisk, R. A. & Powell, T. P. S. *Brain Res.* **52**, 359–362 (1973).
26. Hickey, T. L. *J. comp. Neurol.* **189**, 467–482 (1980).
27. Kalil, R. J. *J. comp. Neurol.* **189**, 483–524 (1980).
28. Guillery, R. W. *J. comp. Neurol.* **144**, 117–130 (1972).
29. LeVay, S., Wiesel, T. N. & Hubel, D. H. *J. comp. Neurol.* **191**, 1–51 (1980).

Monocular deprivation affects X- and Y-cell retinogeniculate terminations in cats

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The X- and Y-cell pathways in cats form two functionally distinct, parallel systems from the retina through the lateral geniculate nucleus (LGN) to the visual cortex^{1–4}. We recently used the technique of intraaxonal injection of horseradish peroxidase (HRP) to demonstrate major differences between X- and Y-cells in their retinogeniculate termination patterns⁵ (compare Figs 1a and 2a). Normally, axons of X-cells innervate geniculate lamina A or A1 (depending on the eye of origin) in narrow zones oriented perpendicular to the lamination. Some X-cells also terminate sparsely (that is, with few boutons) in the medial interlaminar nucleus (MIN), a subdivision of the LGN. Y-cell axons terminate either in laminae C and A (from the contralateral retina) or in lamina A1 (from the ipsilateral retina) in broad zones^{5,6}, and most also terminate densely (with many boutons) in the MIN. We now report that cats raised with monocular lid suture develop abnormal retinogeniculate termination patterns. Many X-cell axons arising from the deprived eye have unusually broad terminal fields in lamina A or A1, and some also densely innervate the MIN. Many Y-cell axons from the deprived eye have dramatically shrunken or absent terminal fields in the A laminae and MIN. These changes constitute the most peripheral effects of monocular deprivation discovered so far, are consistent with previous reports of functional abnormalities among deprived geniculate neurones^{4,7,8} and suggest possible mechanisms by which the visual environment influences neuronal development.

Previously described methods^{5,9,10} were used to obtain results from nine adult cats (>8 months old) raised with continuous monocular deprivation from the first postnatal week. Briefly, recording micropipettes filled with 3% HRP, 0.2 M KCl and 0.05 M Tris were bevelled to 90–120 M Ω . An optic tract axon was first recorded extracellularly and identified as X or Y by standard criteria^{10,11}, including axonal conduction velocity (derived from response latency to electrical stimulation of the optic chiasm) and linearity of spatial summation to visual stimuli (measured by responses to counterphased, sine-wave gratings). Each axon was then impaled, its identity as X or Y was verified intracellularly, and HRP was iontophoresed into it^{5,9}. After sufficient survival time, the brain was sectioned coronally at 100 μ m and reacted with 3-3'-diaminobenzidine plus cobalt chloride¹². The retinas in four animals were dissected free, flat-mounted on slides and reacted with o-dianisidine¹³.

We successfully injected and recovered 9 X- and 11 Y-cell axons from deprived retinas and 11 X- and 7 Y-cell axons from nondeprived retinas. Axonal morphology in lid-sutured animals was also compared with that of 16 X- and 11 Y-cell axons

recovered from normal, adult cats⁵. All axons from the non-deprived eye appeared identical in their termination patterns to axons in normal cats. Among the deprived axons, however, 4 X-cells and 8 Y-cells developed abnormal morphology. Nonetheless, we found no obvious physiological differences between deprived and nondeprived axons.

Monocular deprivation increases the density (number of boutons) and extent of many X-cell terminal fields. Figure 1 illustrates two X-cell axons from the deprived eye. The terminal field in Fig. 1a has apparently normal morphology, being restricted to a narrow zone in lamina A1. As in other normal axons, a thin medialward branch enters the brachium of the superior colliculus but could not be traced to any terminal zone. In contrast, a representative example of a morphologically abnormal X-cell terminal arbor is shown in Fig. 1b. The axon exhibits an abnormally wide terminal field in lamina A1, and it also has many more terminals in the MIN than any normal

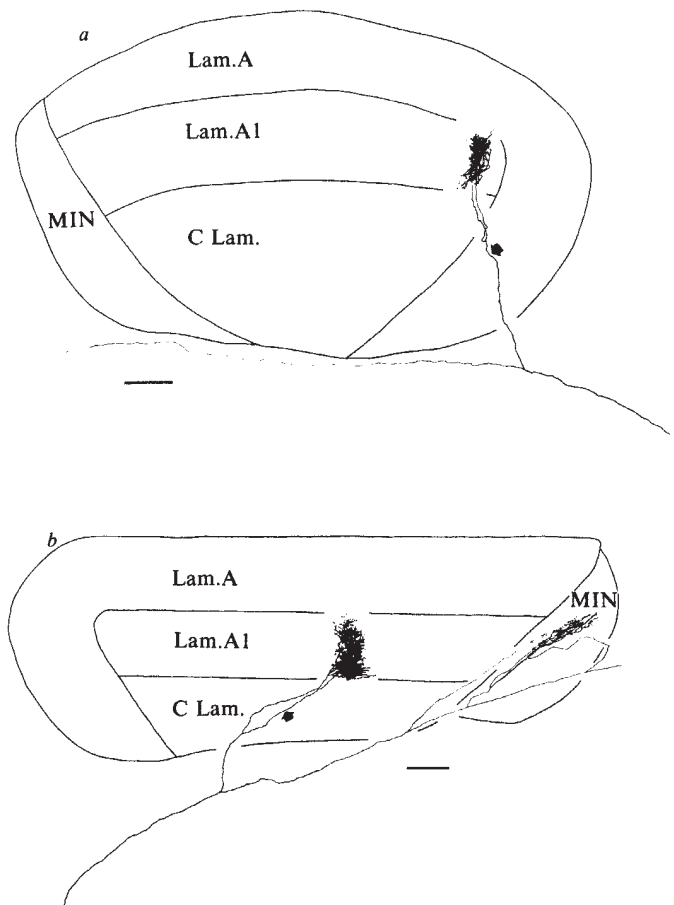


Fig. 1 Geniculate terminal zones of X-cell axons from the deprived eye. Terminal zones of these X- and Y-cell axons were circularly symmetrical in the horizontal plane, and did not show obvious variations in size that could be related to eccentricity or type of centre response (that is, ON or OFF). *a*, Axon from the ipsilateral eye with apparently normal terminal pattern for an X-cell. This axon has 788 terminal boutons in lamina A1, which is within the range of 500–900 boutons for normal X-cell axons in lamina A or A1. Its receptive field was ON centre, had a centre diameter of 1.0°, and was located 44° from the vertical meridian and 23° below the zero horizontal parallel. The axon's conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.8 ms. The axon and its terminal field were reconstructed from 21 serial, 100- μ m thick, coronal sections. Scale bar, 250 μ m. *b*, Axon from the ipsilateral eye with abnormal terminal pattern. The extent of terminal field in either lamina A1 or the MIN exceeds any seen for normal or nondeprived X-cells. Thus, this axon has 1,077 terminal boutons in lamina A1 and 175 boutons in the MIN (compared with 7–31 boutons for normal X-cell axons that innervate the MIN). This axon's receptive field was OFF centre, had a centre diameter of 0.6°, and was located 11° from the vertical meridian and 3° below the horizontal zero parallel. Its conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.7 ms. The axon and its terminal field were reconstructed from 17 serial, 100- μ m thick, coronal sections. Scale bar, 250 μ m.

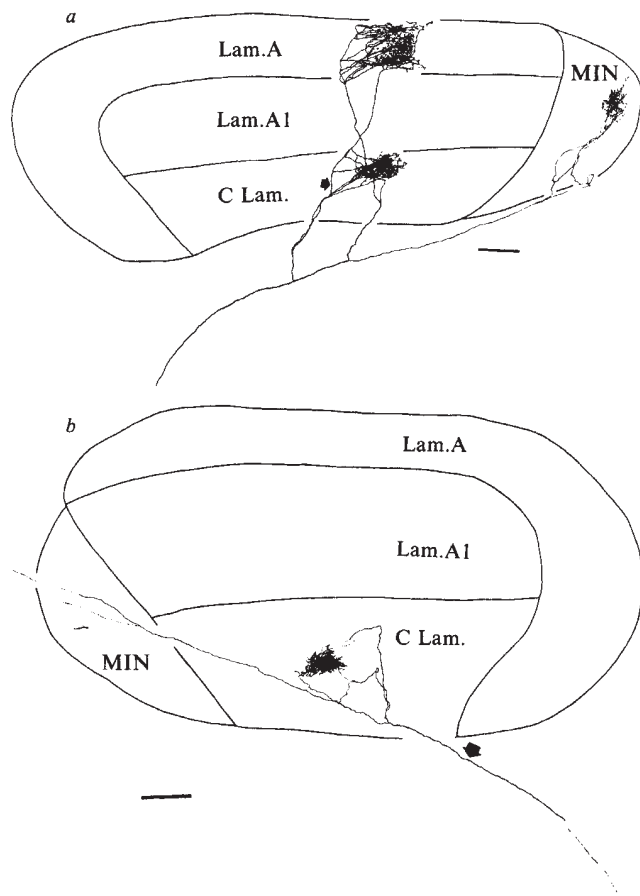


Fig. 2 Geniculate terminal zones of Y-cell axons from the deprived eye. *a*, Axon from the contralateral eye with apparently normal termination pattern for a Y-cell. This axon has 683 terminal boutons in lamina A (the range for normal Y-cell axons in lamina A or A1 is 650–1,400 boutons) and 121 terminal boutons in the MIN (normal range 90–170 boutons). Its receptive field was OFF centre, had a centre diameter of 1.3° , and was located 4° from the vertical meridian and 7° below the zero horizontal parallel. The axonal conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.5 ms. The axon and its terminal field were reconstructed from 19 serial, 100- μ m thick, coronal sections. Scale bar, 250 μ m. *b*, Axon from the contralateral eye with abnormal terminal pattern. The axon terminates densely only in the C-laminae, has no terminal boutons in the A-laminae and sparsely innervates the MIN with only 6 boutons. This contrasts sharply with the normal pattern described in the text. This axon's receptive field was OFF centre, had a centre diameter of 1.6° , and was located 7° from the vertical meridian and 40° below the zero horizontal parallel. Its axonal conduction latency from the optic chiasm to the recording and injection site (arrow) was 0.4 ms. The axon and terminal field were reconstructed from 18 serial, 100- μ m thick, coronal sections. Scale bar, 250 μ m.

X-cell axon seen to date. Figure 3 summarizes some of these phenomena for arbors in the A-laminae.

Monocular deprivation affects Y-cells even more dramatically than it does X-cells, but in a different way. Figure 2*a* shows a normal Y-cell terminal field from the deprived retina. The axon terminates densely in laminae A and C, plus the MIN. In contrast, Fig. 2*b* illustrates an example of a deprived Y-cell axon that terminates densely in the C-laminae, sparsely in the MIN and not at all in the A-laminae. In normal cats, every Y-cell axon found terminates densely in lamina A or A1, and the majority that terminate in the MIN do so densely there^{5,6}. Of six deprived Y-cell axons from the contralateral eye, four innervate only the C-laminae and do not terminate in lamina A. Four additional deprived Y-cell axons (one contralateral, three ipsilateral) have terminal fields in the A-laminae that are narrower and with fewer boutons than normal. Thus, deprived Y-cell terminals are most affected in the binocular segment of the A-laminae (Fig. 3), less so in the MIN, and seem normal in the C-laminae. The clearest effect of monocular lid suture on retinogeniculate Y-cell terminations, however, is that many

contralateral Y-cell axons innervate only the C-laminae and do not innervate lamina A. If these four axons that fail to innervate lamina A are removed from the comparison in Fig. 3, the remaining Y-cell terminal fields are only marginally smaller than those of the normal and nondeprived Y-cells. More data are needed to determine whether the effects of deprivation are continuous such that some Y-cell terminal zones in the A-laminae are missing, some smaller than normal, and some normal, or whether the effects cause complete elimination of some Y-cell terminal fields with no effect on others.

We are confident that the abnormally small terminal fields seen in many deprived Y-cell axons are not due to incomplete or poor staining. These axons were darkly stained, as were the nondeprived and normal axons, and each axonal branch could be traced to a terminal bouton. Also, the same tissue often included axons with normal and abnormal morphology.

Figure 4 shows the retrograde labelling obtained for the somata of two identified axons that were filled with HRP. Such labelling was obtained for four X- and four Y-cells in deprived retinas and three X- and five Y-cells in nondeprived retinas. X-cell somata were consistently smaller than Y-cell somata¹⁴, but no obvious size differences could be seen between the deprived and nondeprived somata despite large changes in the terminal field extents of the former. However, such comparisons are complicated by differences in retinal eccentricity^{15,16}.

Many studies have established that monocular deprivation causes abnormalities to develop in the cat's central visual pathways⁴. The expanded X-cell and reduced or absent Y-cell retinogeniculate projections described here constitute the most peripheral morphological effects of deprivation seen so far. Furthermore, although our study seems inconsistent with a recent ultrastructural study¹⁷ that reported normal numbers and structure for retinal synapses in the deprived A-laminae,

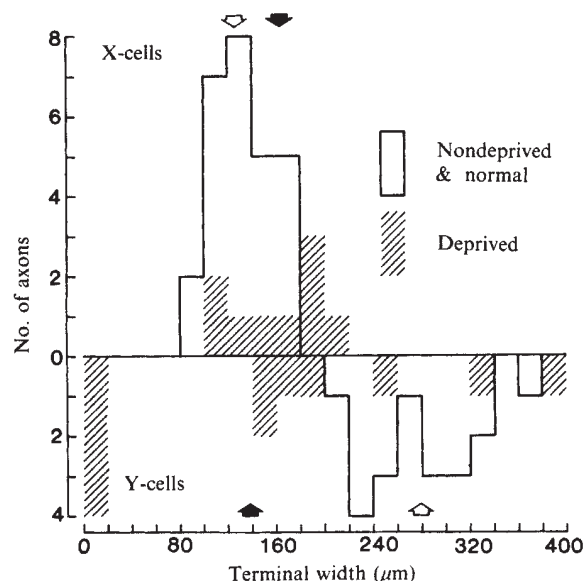


Fig. 3 Maximum widths of terminal zones in the A-laminae for X- and Y-cell axons with receptive fields in the binocular segment. The widths were measured along a plane parallel to the lamination. Because this is the plane of circular symmetry for these terminal fields and there is no effect of eccentricity or centre type on their size (see Fig. 1 legend), it seems unlikely that differences in terminal field widths result from histological artefacts. Data for nondeprived axons and axons from normally reared cats were pooled, as they are morphologically indistinguishable. Open arrows denote mean widths for normal and nondeprived axons; closed arrows, for deprived axons. Terminal zones for deprived X-cell axons are larger than those for nondeprived or normal X-cell axons ($P < 0.05$ on a Mann-Whitney *U*-test for either comparison). These zones for deprived Y-cell axons are smaller than those for nondeprived or normal Y-cell axons ($P < 0.01$ on a Mann-Whitney *U*-test for either comparison). However, if the four contralateral Y-cell axons that do not innervate lamina A are omitted from the comparison, the difference between deprived and nondeprived or normal Y-cell terminal widths in the A-laminae is less significant ($0.05 < P < 0.1$ on a Mann-Whitney *U*-test).

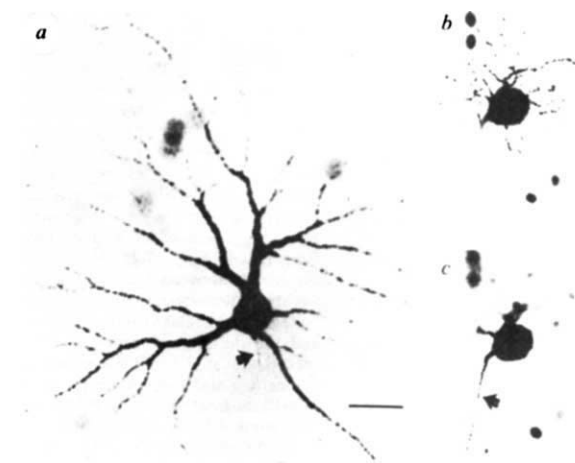


Fig. 4 Photomicrographs of retinal ganglion cells labelled retrogradely following HRP injection into their axons. Each ganglion cell was identified unambiguously by comparing its location relative to retinal blood vessels with plots of the vessels and receptive field made during the recording. *a*, Y-cell in nondeprived retina. The neurone's receptive field was OFF centre, had a centre diameter of 2.6° , and was located 17° from the vertical meridian and 45° below the zero horizontal parallel. The morphology of this Y-cell is characteristic of α cells¹⁴⁻¹⁶. The arrow points to the axon. Scale bar, $50\ \mu\text{m}$ and applies as well to *b* and *c*. *b*, *c*, Two focal planes of same field of view showing an X-cell in nondeprived retina. The neurone's receptive field was OFF centre, had a centre diameter of 0.9° , and was located 20° from the vertical meridian and 5° below the zero horizontal parallel. The morphology of this X-cell is characteristic of β cells¹⁴⁻¹⁶. The focus is adjusted for the dendrites in *b* and for the soma and axon (arrow) in *c*.

the two sets of data may actually be compatible. Prior evidence suggests that retinal X-cells outnumber Y-cells by roughly 10 to 1 (refs 15, 16). Even with roughly twice as many boutons from Y-cell axons as from X-cell axons⁵, retinogeniculate synapses in the A-laminae arising from X-cells probably outnumber substantially those from Y-cells. It is impossible to distinguish between X- and Y-cell retinogeniculate synapses in conventional ultrastructural material¹⁸. Thus, if one were to study these synapses in deprived laminae without knowledge of X- and Y-cell identity, a fairly substantial loss of terminals from the less numerous Y-cell axons with a slight expansion of terminals from more numerous X-cell axons might result in ultrastructure that appeared fairly normal. On the other hand, our data are consistent with both the loss of recorded geniculate Y-cells from deprived laminae⁷ and the finding that many deprived geniculate neurones that normally accept only Y-cell input from the retina accept or retain X-cell input instead⁸.

The abnormal terminal arbors we have described suggest that deprivation might cause retinogeniculate X-cell connections to expand at the expense of Y-cell connections by some sort of competitive interaction during development. X-cell axons normally seem to innervate the lateral geniculate nucleus before Y-cell axons do^{19,20}. Perhaps, as in many developing pathways²¹⁻²³, the immature X-cell pathway is exuberant and is 'pruned back' in competitive interactions with the later-developing Y-cell inputs. The ability of Y-cell axons to dislodge the already-present X-cell axons may be severely reduced during visual deprivation. Consistent with this hypothesis is our observation that deprived Y-cell terminals are most severely affected in the A-laminae, where geniculate X-cells are concentrated. One means of testing this hypothesis would be to study the termination patterns of X- and Y-cell retinogeniculate axons in developing kittens.

Regardless of the developmental mechanisms, our evidence clearly shows that the visual environment can influence the formation of retinogeniculate terminations. Early visual deprivation causes abnormalities in these connections, which most seriously affect the Y-cell pathway. This might well represent the neural basis of amblyopia seen in monocularly deprived cats, as the Y-cell pathway may have a crucial role in normal vision²⁴.

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1. Rodieck, R. W. A. *Rev. Neurosci.* **2**, 193-225 (1979).
2. Stone, J., Dreher, B. & Leventhal, A. *Brain Res. Rev.* **1**, 345-394 (1979).
3. Lennie, P. *Vision Res.* **20**, 561-594 (1980).
4. Sherman, S. M. & Spear, P. D. *Physiol. Rev.* **62**, 738-855 (1982).
5. Sur, M. & Sherman, S. M. *Science* **218**, 389-391 (1982).
6. Bowling, D. B. & Michael, C. R. *Nature* **286**, 899-902 (1980).
7. Sherman, S. M., Hoffman, K.-P. & Stone, J. *J. Neurophysiol.* **35**, 532-541 (1972).
8. Friedlander, M. J., Stanford, L. R. & Sherman, S. M. *J. Neurosci.* **2**, 321-330 (1982).
9. Friedlander, M. J., Lin, C.-S., Stanford, L. R. & Sherman, S. M. *J. Neurophysiol.* **46**, 80-129 (1981).
10. Hoffmann, K.-P., Stone, J. & Sherman, S. M. *J. Neurophysiol.* **35**, 518-531 (1972).
11. Hochstein, S. & Shapley, R. M. *J. Physiol., Lond.* **262**, 237-264 (1976).
12. Adams, J. C. *Neuroscience* **2**, 141-145 (1977).
13. deOlmos, J. S. *Expl Brain Res.* **29**, 541-551 (1977).
14. Boycott, B. B. & Wässle, H. *J. Physiol., Lond.* **240**, 397-419 (1974).
15. Wässle, H., Peichl, L. & Boycott, B. B. *Proc. R. Soc. B* **212**, 157-175 (1981).
16. Wässle, H., Boycott, B. B. & Illing, R.-B. *Proc. R. Soc. B* **212**, 177-195 (1981).
17. Winfield, D. A. & Powell, T. P. S. *Proc. R. Soc. B* **210**, 197-210, 211-234 (1980).
18. Wilson, J. R., Friedlander, M. J. & Sherman, S. M. *Soc. Neurosci. Abstr.* **6**, 583 (1980).
19. Daniels, J. D., Pettigrew, J. D. & Norman, J. L. *J. Neurophysiol.* **41**, 1373-1393 (1978).
20. Torrealba, F., Guillery, R. W., Eysel, U., Polley, E. H. & Mason, C. A. *J. Comp. Neurol.* (in the press).
21. Purves, D. & Lichtman, J. R. *Science* **210**, 153-157 (1980).
22. Rakic, P. *Nature* **261**, 467-471 (1976).
23. Ivy, G. O. & Killackey, H. P. *J. Neurosci.* **2**, 735-743 (1982).
24. Sherman, S. M. in *Changing Concepts of the Nervous System* (eds Morrison, A. R. & Strick, P. L.) 337-359 (Academic, New York, 1982).

Action potential repolarization may involve a transient, Ca^{2+} -sensitive outward current in a vertebrate neurone

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Repolarization of the action potential in squid axon¹ and several types of neurones²⁻⁴ involves a voltage-activated potassium (K^+) current. Voltage clamp analysis has demonstrated that this current has rapid activation kinetics^{1,3-5}. In several neuronal types, the same technique has also revealed a slowly activated K^+ current that is calcium (Ca^{2+})-sensitive^{3,5-10}. This slow Ca^{2+} -activated K^+ current is the major current underlying the late, slower portion of the after-hyperpolarization following an action potential¹¹⁻¹⁴. In several muscle types, fast, transient Ca^{2+} -dependent K^+ currents have been described¹⁵⁻¹⁷ which may contribute to repolarization of the action potential. Rapidly activating, Ca^{2+} -dependent K^+ currents have been observed in sympathetic neurones of the bullfrog and it has been suggested that they contribute to action potential repolarization of those neurones^{8,9,18}. We have studied the membrane currents in bullfrog sympathetic neurones using voltage clamp methods and report here a transient outward current that appears to be composed of two separate currents. One of those currents is a transient, Ca^{2+} -sensitive outward current as indicated by a significant reduction of the current by treatments that reduce or block Ca^{2+} entry (Mn^{2+} , Cd^{2+} , Co^{2+} , Mg^{2+} or Ca^{2+} -free Ringer). Such treatments also decreased the rate of action potential repolarization. The results suggest that this current is involved in repolarization of the action potential and consequently may regulate Ca^{2+} entry into the neurone during spike activity.

The IXth and Xth paravertebral sympathetic ganglia of bullfrog (*Rana catesbeiana*) were excised, pinned in a Plexiglass recording chamber and continuously superfused with a Ringer's solution of the following composition (mM): NaCl 117; KCl 2.5; CaCl_2 1.8; HEPES 1 (pH 7.3). Most preparations were

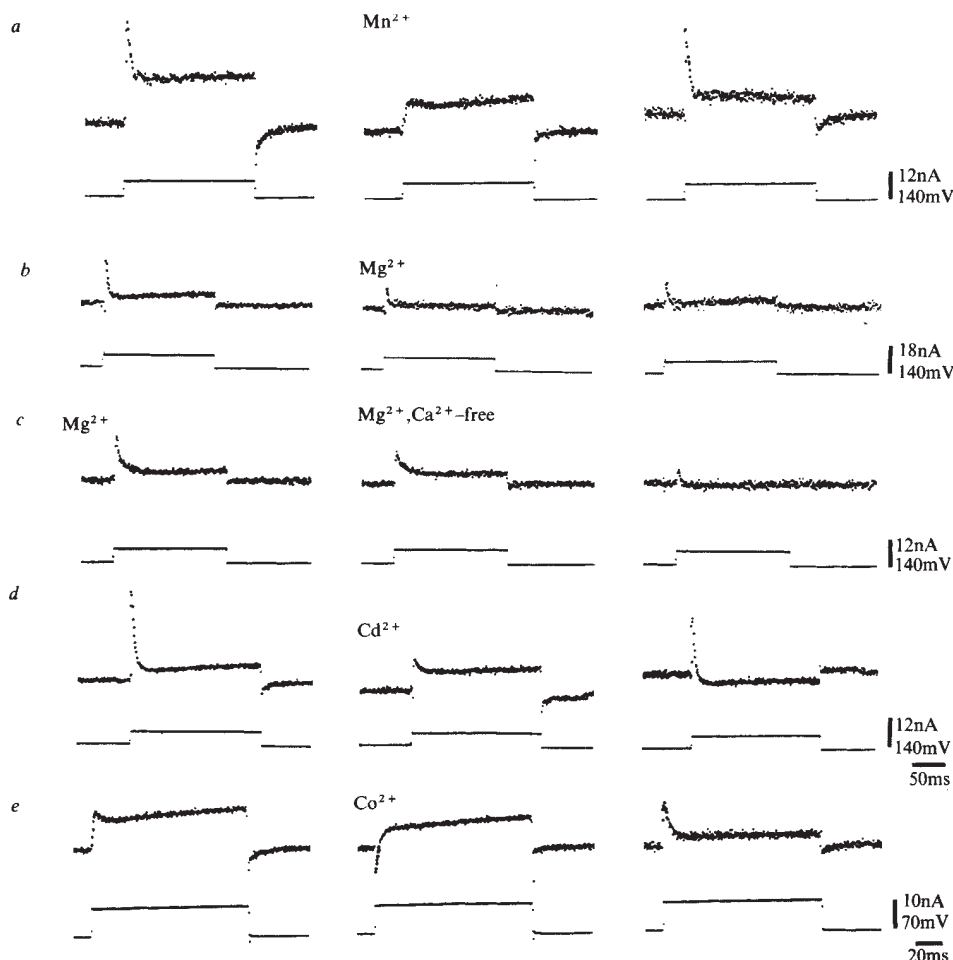


Fig. 1 Effect of divalent cations on both transient and late outward currents. Voltage-clamp records of currents activated by a depolarizing step of membrane voltage in five separate experiments (a-e). Left: currents activated by depolarizing step in normal Ringer's solution, except c in which 8 mM Mg^{2+} was added to the normal Ringer. Middle: effect of changing divalent cations on the currents activated by a depolarizing step to the same voltage; the divalent cations illustrated and the duration of exposure to the cations are: a, 2.5 mM manganese for 9 min; b, 8 mM magnesium for 4 min; c, calcium-free with 10 mM Mg^{2+} for 6.5 min; d, 0.5 mM cadmium for 5 min; e, 3 mM cobalt for 16 min. Right: difference current obtained by subtracting the currents recorded with altered divalent cations (middle) from control currents in normal Ringer (left). In the difference record in d, the inward current that follows the transient outward current resulted from an increased leak current which occurred in Cd^{2+} . An increased leak was not observed in the presence of the other divalent cations. In a-e the depolarizing step was to -15 mV. The holding potentials were: a, -92 mV; b, -82 mV; c, -78 mV; d, -80 mV; e, -85 mV. Experiments were conducted using the two-microelectrode voltage-clamp technique, as described previously¹⁹. The records are from data stored by a PDP 11/03 micro-computer. The current signal was filtered for frequencies above 1 or 3 kHz, which is similar to the settling time of the clamp; therefore the peak amplitude of fast current may be underestimated.

treated with 1.0% trypsin for 5-7 min followed by a 10-min exposure to trypsin inhibitor before final desheathing of the ganglion. Large neurones (>50 μ m) were selected for impalement. Microelectrodes were filled with 3 M KCl and had an electrical resistance of 25-40 M Ω . Two microelectrodes were used for voltage-clamp experiments as described previously¹⁹; a single microelectrode was used for recording the action potential.

Both the early and late currents activated by depolarizing steps of membrane voltage and the effect of various treatments that reduce Ca^{2+} entry on those currents are illustrated in Fig. 1. The records on the left in Fig. 1 show the currents activated by depolarizing voltage steps in five different experiments (a-e). At the initiation of the voltage step there was a quickly activating, transient outward current that decayed rapidly. It was followed by a slow outward current that has been described previously⁷⁻¹⁰. The middle records illustrate the effect of 2.5 mM manganese (a), 8 mM magnesium (b), Ca^{2+} -free Ringer (c), 0.5 mM cadmium (d) and 3 mM cobalt (e). The transient outward current was significantly reduced by each of these treatments. The records on the right are the difference currents, which were obtained by subtracting the currents recorded in the altered divalent cation Ringer (middle) from the currents recorded in normal Ringer's solution (left). The right-hand records thus show the currents that were sensitive to either the addition of a Ca^{2+} blocking divalent cation or the omission of Ca^{2+} from the Ringer's solution.

Figure 2 shows the transient outward current recorded at a rapid sampling rate. Voltage control of the soma was achieved before the current reached peak amplitude. The development of this current with a depolarizing voltage step from -59 to -21 mV is shown in Fig. 2a. Following the return of membrane potential to -49 mV, a fast outward tail can be observed. In

Fig. 2b, an inward current is seen before the transient outward current. Figure 2c shows two pairs of currents recorded from the same cell before and during exposure to 3 mM Co^{2+} . In normal Ringer (Fig. 2ci), the transient outward current developed quickly with the peak amplitude depending on the holding potential (-76 and -104 mV). In the Ringer containing Co^{2+} (Fig. 2cii), an inward current was revealed followed by a more slowly developing, but early, outward current. The divalent cation-sensitive component of the transient outward current was a labile current. It seemed to be present only in cells that had resting potentials > -40 mV and spike amplitudes > 80 mV following impalement with two electrodes. The amplitude of the transient outward current declined slowly with time under clamp, making recovery from a divalent cation block difficult to demonstrate. We have, however, observed recovery of both the transient and slow outward currents following exposure to Mg^{2+} , which washes out relatively quickly.

Figure 3 illustrates the effect of membrane holding potential on the activation of the transient outward current. Figure 3ai shows examples of the currents activated in normal Ringer's solution from membrane holding potentials from left to right of -28, -37, -55 and -92 mV. Figure 3b is a graph of the amplitude of the transient outward current as a function of membrane holding potential. In normal Ringer's solution, the current was activated over the range of holding potentials tested, from -105 mV to -23 mV. The amplitude of this transient current decreased as membrane holding potential was made more positive, suggesting a voltage-dependent inactivation process. The strong dependence of the transient current on holding voltage is not characteristic of the slower, 'steady-state' currents. In most cells there was a decrease in the slope of the inactivation curve at membrane holding potentials near both -60 mV and -90 mV (Fig. 3b). The currents activated

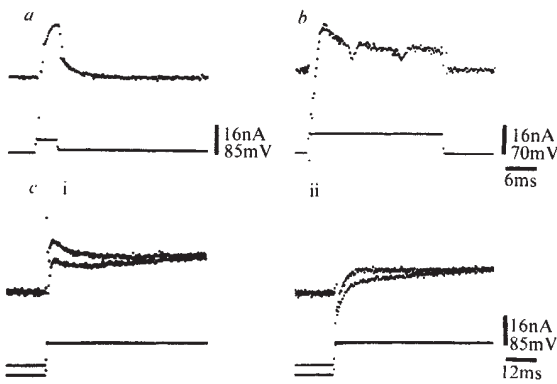


Fig. 2 Transient outward current recorded at a rapid sampling rate. *a*, Transient outward current activated by a voltage step from -59 to -21 mV. Shortly after the current peaked, membrane potential was stepped to -49 mV; this was accompanied by an outward tail current (filtered at 1 kHz). *b*, Response of a different cell clamped at -59 mV and stepped to -15 mV (filtered at 10 kHz). In this case, the transient outward current was preceded by an inward current. The two inward current events riding on the decaying outward current are presumably from axonal spikes. *c*, Current records from the same cell before (ci) and during (cii) exposure to a Ringer's solution containing 3 mM Co^{2+} . The membrane was voltage clamped at holding potentials of -76 and -104 mV and stepped to -15 mV to activate the outward current. Filtering frequency was 3 kHz.

from the same series of membrane holding potentials in the presence of 2.5 mM Mn^{2+} are shown in Fig. 3a. The amplitude of the transient current in the presence of Mn^{2+} is plotted in Fig. 3b. In the Mn^{2+} containing Ringer, a transient outward current was only apparent at membrane holding potentials negative to -60 mV. In addition, the shape of the inactivation curve in Mn^{2+} differed from the curve in normal Ringer, with a single, smooth rising phase between -60 and -80 mV and a distinct flattening at more negative potentials. The voltage-dependent inactivation characteristics of this transient outward current are similar to those of the A-current, first identified in molluscan neurones²⁰. Similar results were obtained with Ringer's solutions containing 0.5 mM Cd^{2+} , 3.0 mM Co^{2+} , and with Ca^{2+} -free Ringer. Figure 3a.ii also shows that in the presence of 2.5 mM Mn^{2+} , an early inward current was revealed as in Fig. 2cii. This inward current accounts for the apparent reversal of the transient outward current in Fig. 3b. The inward current was partially blocked by 10^{-5} M tetrodotoxin (not shown) and is presumably a Na^{+} current.

The effect on action potential repolarization of treatments that reduce Ca^{2+} entry are illustrated in Fig. 4. In the presence of a Ringer's solution containing 3 mM Mn^{2+} , the rate of action potential repolarization was slowed, resulting in an increased spike duration (Fig. 4a, upper traces). In addition, the amplitude of the after-hyperpolarization following the action potential was reduced. These changes can be seen more clearly in the electronically differentiated records (lower traces). Figure 3b shows the effect of a Ca^{2+} -free Ringer on the generation of the action potential. In these records, successive traces were superimposed during both the wash-in and the wash-out of the Ca^{2+} -free Ringer, showing clearly both the decreased rate of action potential repolarization and the reduction of the after-hyperpolarization. Similar effects were also observed with 0.5 mM Cd^{2+} .

The reduction of the transient outward current by a Ca^{2+} -free Ringer indicates that at least part of the current is Ca^{2+} -sensitive. The similar effect of Mn^{2+} , Cd^{2+} , Co^{2+} and Mg^{2+} suggests that these divalent cations suppress this Ca^{2+} -sensitive component of the transient current. The divalent cations used have been found to reduce or block the entry of Ca^{2+} in many cell types²¹. In bullfrog sympathetic neurones, Cd^{2+} has been shown to antagonize the Ca^{2+} current recorded in voltage-clamp experiments¹⁰. Moreover, in experiments using the Ca^{2+} -sensitive dye Arsenazo III to detect intracellular Ca^{2+} in these neurones²², Mn^{2+} has been found to block the entry of Ca^{2+} resulting from a train of action potentials (S. J. Smith, A. B.

MacD., and F.F.W., in preparation). The effect of membrane holding potential indicates that the Ca^{2+} -sensitive transient outward current has characteristics that differ significantly from the characteristics of the transient outward current recorded in the presence of Mn^{2+} , Cd^{2+} , Co^{2+} or Mg^{2+} , or with omission of Ca^{2+} from the Ringer's solution which suggests the presence of two separate currents. The presence of two, transient outward currents that both decay rapidly, one of which may be Ca^{2+} -dependent, has also been identified in cardiac Purkinje fibres²³.

A Ringer's solution containing 10 mM tetraethylammonium (TEA) or 5 mM Ba^{2+} blocks virtually all of the transient outward current⁹. TEA inhibits the voltage-activated or rectifier K^{+} current^{6,24} and can also block Ca^{2+} -sensitive K^{+} currents²⁵⁻²⁷. Ba^{2+} has been found to block several K^{+} currents in neurones including Ca^{2+} -sensitive^{27,28}, delayed rectifier^{29,30}, and M-currents^{7,9,31}. Inhibition of the transient outward current in bullfrog sympathetic neurones by TEA or Ba^{2+} suggests that both the Ca^{2+} -sensitive transient outward current and the transient outward current that is not Ca^{2+} -sensitive are carried by K^{+} . Further evidence that they are carried by K^{+} is the observation that the amplitude of the transient outward current decreased in a Ringer containing 25 mM KCl. Similar fast K^{+}

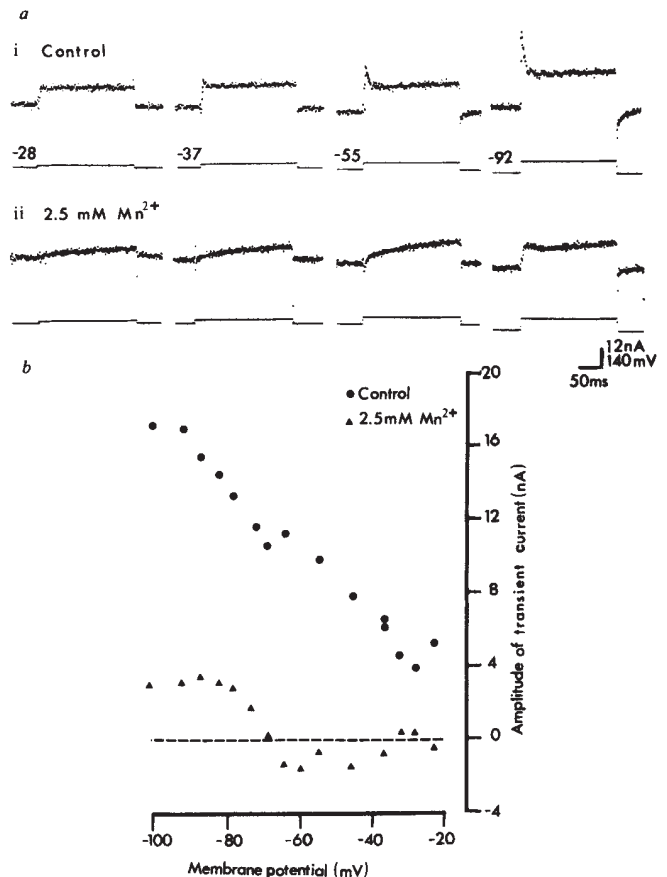


Fig. 3 Effect of membrane potential on amplitude of the transient outward current. *a*, Membrane voltage was held at a given potential for 8 s, stepped to an activation voltage of -15 mV for 200 ms, then returned to a new, more negative holding potential. (Paired pulse experiments showed that holding at a negative holding potential for more than 500 ms was necessary for recovery from inactivation.) *ai*, Effect of membrane potential on currents activated by a depolarizing step to -15 mV in normal Ringer's solution; the membrane holding voltage before each step is labelled on the voltage trace. *a.ii*, Effect of 2.5 mM Mn^{2+} on outward currents activated by the same depolarizing steps from the same holding potentials in the same cell as *ai*. *b*, Graph of relationship between amplitude of the transient outward current (nA) and membrane holding potential (mV) before depolarizing step to -15 mV in normal Ringer (●) and a Ringer containing 2.5 mM Mn^{2+} (▲). Amplitude of the transient outward current was measured as the difference between the transient outward current peak and the baseline current, minus the leak current. Leak current was calculated by dividing the voltage difference of the step by the slope resistance between -65 and -90 mV. Experiments conducted using two microelectrode voltage-clamp technique¹⁹.

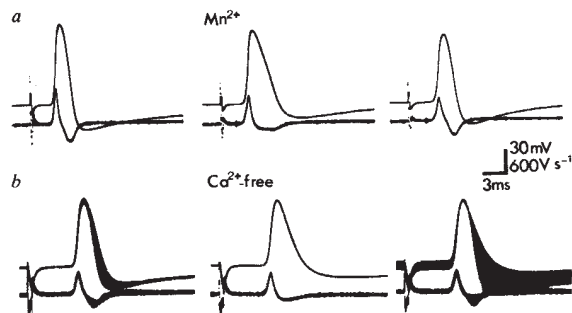


Fig. 4 Effect of divalent cations on action potential repolarization. *a*, Effect of 3 mM Mn^{2+} on the action potential. Left: action potential recorded in normal Ringer's solution. Middle: action potential recorded after superfusion for 6 min with Ringer containing 3 mM Mn^{2+} . Right: action potential recorded 13 min after beginning Mn^{2+} wash-out. Top trace: voltage recording of action potential. Bottom trace: electronically differentiated record of action potential. Since the addition of Mn^{2+} depolarized the cell, the membrane potential was offset to the control resting potential (-55 mV in this cell) using a bridge circuit. *b*, Effect of Ca^{2+} -free Ringer containing 6 mM Mg^{2+} on the action potential. Left: superimposed action potentials recorded initially in normal Ringer and during wash-in with Ca^{2+} -free Ringer containing 6 mM Mg^{2+} . Middle: single action potential recorded after superfusion for 10 min with Ca^{2+} -free Ringer containing 6 mM Mg^{2+} . Right: superimposed action potentials during wash-out of the Ca^{2+} -free Ringer. The resting membrane potential of this cell was -60 mV. Top trace: voltage recording of action potential. Bottom trace: electronically differentiated record of action potential. Action potentials were elicited by antidromic stimulation of the IXth or Xth spinal nerves. Records were photographed from oscilloscope trace.

currents which appear to be Ca^{2+} -sensitive or partially Ca^{2+} -sensitive have been identified in cardiac Purkinje fibres^{17,32}, crab muscle¹⁶ and guinea pig myometrium¹⁵.

The transient outward current reported here seems to be different from another fast, Ca^{2+} -activated K^+ current observed in bullfrog sympathetic neurones. Adams *et al.*¹⁸ recently described a fast, Ca^{2+} -activated, voltage-sensitive K^+ current with an activation time constant of about 15–20 ms. The various methods used to estimate the time constant in their study involved holding the membrane potential at -10 mV. The transient outward current studied in the experiments reported here also peaks rapidly, in less than 5 ms with pulses to -10 mV; however, it inactivates rapidly following activation and thus presumably does not contribute to the currents measured in the study of Adams *et al.* As the mechanisms underlying the different properties of these two currents are unclear, the relationship between these two Ca^{2+} -sensitive outward currents is a question for future investigation.

In cat motoneurone, a fast K^+ current has been studied which is blocked by Co^{2+} , Mn^{2+} , Ni^{2+} and TEA³³. However, these treatments did not appreciably broaden the directly activated action potential. In contrast, a decreased rate of action potential repolarization was observed in bullfrog sympathetic neurones in Ca^{2+} -free Ringer or in the presence of Ca^{2+} -blocking divalent cations. This suggests that the Ca^{2+} -sensitive transient outward current contributes to repolarization of the action potential in these vertebrate neurones. It is also possible that this current may limit the entry of Ca^{2+} into the neurone during spike activity. Since a voltage-sensitive Ca^{2+} current is activated during membrane depolarization^{10,22,34}, an outward current that is rapidly activated by Ca^{2+} entry would rapidly repolarize the cell thus inhibiting further Ca^{2+} entry.

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- Hodgkin, A. L. & Huxley, A. F. *J. Physiol., Lond.* **117**, 500–544 (1952).
- Aldrich, R. W., Getting, P. A. & Thompson, S. H. *J. Physiol., Lond.* **291**, 531–544 (1979).
- Barrett, E. F., Barrett, J. N. & Crill, W. E. *J. Physiol., Lond.* **304**, 251–276 (1980).
- Connor, J. A. & Stevens, C. F. *J. Physiol., Lond.* **213**, 31–53 (1971).
- Meech, R. W. & Standen, N. B. *J. Physiol., Lond.* **249**, 211–239 (1975).
- Thompson, S. H. *J. Physiol., Lond.* **265**, 465–488 (1977).
- MacDermott, A. B. & Weight, F. F. *Fedn Proc.* **39**, 2074 (1980).
- Constanti, A., Adams, P. R. & Brown, D. A. *Soc. Neurosci. Abstr.* **7**, 14 (1981).

- Weight, F. F. & MacDermott, A. B. in *Physiology and Pharmacology of Epileptogenic Phenomena* (eds Klee, M. R., Lux, H. D. & Speckmann, E. J.) 227–233 (Raven, New York, 1982).
- Adams, P. R., Brown, D. A. & Constanti, A. in *Physiology and Pharmacology of Epileptogenic Phenomena* (eds Klee, M. R., Lux, H. D. & Speckmann, E. J.) 175–187 (Raven, New York, 1982).
- Minota, S. *Jap. J. Physiol.* **24**, 501–512 (1974).
- Barrett, E. F. & Barrett, J. N. *J. Physiol., Lond.* **255**, 737–774 (1976).
- Busis, N. A. & Weight, F. F. *Nature* **263**, 434–436 (1976).
- McAfee, D. A. & Yarowsky, P. J. *J. Physiol., Lond.* **290**, 507–523 (1979).
- Vassort, G. *J. Physiol., Lond.* **252**, 713–734 (1975).
- Mounier, Y. & Vassort, G. *J. Physiol., Lond.* **251**, 609–625 (1975).
- Siegelbaum, S. A. & Tsien, R. W. *J. Physiol., Lond.* **299**, 485–506 (1980).
- Adams, P. R., Constanti, A., Brown, D. A. & Clark, R. B. *Nature* **296**, 746–749 (1982).
- MacDermott, A. B., Connor, E. A., Dionne, V. E. & Parsons, R. L. *J. gen. Physiol.* **75**, 39–60 (1980).
- Connor, J. A. & Stevens, C. F. *J. Physiol., Lond.* **213**, 21–30 (1971).
- Hagiwara, S. & Byerly, L. A. *Rev. Neurosci.* **4**, 69–125 (1981).
- Smith, S. J., MacDermott, A. B. & Weight, F. F. *Neurosci. Abstr.* **7**, 15 (1981).
- Coraboeuf, E. & Carmeliet, E. *Pflügers Arch. ges. Physiol.* **392**, 352–359 (1982).
- Aldrich, R. W., Getting, P. A. & Thompson, S. H. *J. Physiol., Lond.* **291**, 507–530 (1979).
- Hermann, A. & Gorman, A. L. F. *J. gen. Physiol.* **78**, 87–110 (1981).
- Meech, R. W. & Standen, N. B. *J. Physiol., Lond.* **249**, 211–239 (1975).
- Connor, J. A. *J. Physiol., Lond.* **286**, 41–60 (1979).
- Meech, R. W. *Ann. Rev. Biophys. Bioengng* **7**, 1–18 (1978).
- Armstrong, C. M. & Taylor, S. R. *Biophys. J.* **30**, 473–488 (1980).
- Eaton, D. C. & Brodwick, M. S. *J. gen. Physiol.* **75**, 727–750 (1980).
- Constanti, A., Adams, P. R. & Brown, D. A. *Brain Res.* **206**, 244–250 (1981).
- Kenyon, J. L. & Gibbons, W. R. *J. gen. Physiol.* **73**, 117–138 (1979).
- Schwandt, P. C. & Crill, W. E. *J. Neurophysiol.* **46**, 1–16 (1981).
- Adams, P. R. in *Advances in Physiological Sciences* Vol. 4 (ed. Salanki, J.) 135–138 (Pergamon, New York, 1981).

Generation of mucosal mast cells is stimulated *in vitro* by factors derived from T cells of helminth-infected rats

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The connective tissue of rats, and several other species of mammals, contains two distinct types of mast cells that differ in morphology, histochemical staining properties and location¹. One type, frequently called the normal connective tissue mast cell, can be obtained in nearly homogeneous preparation from a mixed cell population in the peritoneal cavity and forms the basis of our knowledge of mast cells. The other type is referred to as the mucosal mast cell because in normal rats it has been observed only in mucosal tissue. Infection with helminth parasites induces an extensive accumulation of mast cells and eosinophils in the tissues^{1,2}, and parasites of mucous surfaces, in particular, stimulate a rapid hyperplasia of mucosal mast cells^{3,4}. However, the origin of mucosal mast cells, and their relationship to the connective tissue mast cells is uncertain. We now show that lymphocytes of helminth-infected rats, on *in vitro* stimulation with specific antigen, release factors causing pronounced mucosal mastocytosis in normal rat bone marrow cultures.

Conditioned media capable of causing the proliferation of mast cells were derived from cultures of antigen-stimulated mesenteric lymph node cells of rats infected with the nematode *Nippostrongylus brasiliensis*. Figure 1 illustrates that the medium from such cultures added to normal rat bone marrow cultures encourages cell growth and enriches for mast cells. The conditioned medium also contains some growth stimulating activity for neutrophils, macrophages and eosinophils (not shown). However, restimulation and refeeding leads to cultures containing greater than 90% mast cells. By contrast, the culture

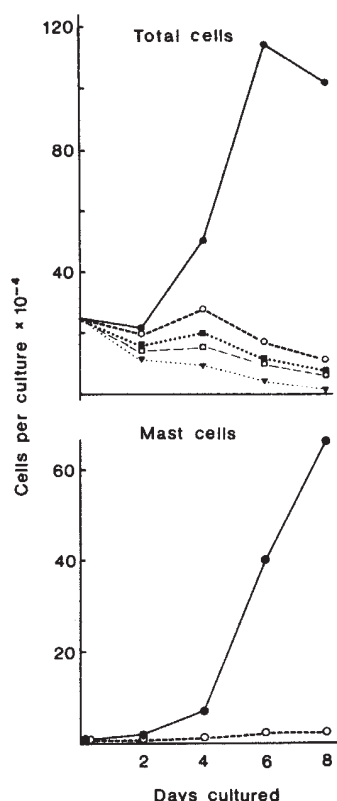


Fig. 1 Growth of bone marrow cells in the presence of culture fluids from antigen-stimulated mesenteric lymph node cells (MLN) of rats infected with *N. brasiliensis*. Shown above, in the upper section, total cells in bone marrow cultures (mean of 3) with added supernatant from: antigen-stimulated infected rat MLN (●); antigen-unstimulated infected rat MLN (○); antigen-stimulated (■) and unstimulated normal rat MLN (□); and unconditioned medium containing Nb antigen only (▼). In the lower section, mast cells in the first two of the above cultures. The bone marrow culture technique was adapted from the method of Sumner *et al.*²³. Normal bone marrow cells from (LIS × BN) F₁ rats suspended at $2.5 \times 10^5 \text{ ml}^{-1}$ in Iscove's medium (Gibco) with 20% horse serum were cultured in 35 mm tissue culture dishes at 37 °C in a humidified incubator flushed with 5% CO₂ in air. Any effect of small amounts of endogenous growth stimulating activity is not detected at this low cell density: these cultures thus provide a simple system for the assay of exogenous growth stimulating activity. Twenty per cent conditioned medium, obtained by culturing syngeneic mesenteric lymph node cells from normal or infected rats in the presence of *N. brasiliensis* antigen, was added to the cultures. Rats were infected with 4,000 *N. brasiliensis* larvae²⁴ and mesenteric lymph nodes were removed between 12 and 30 days after infection. The lymph node cells were suspended at $4 \times 10^6 \text{ ml}^{-1}$ in Iscove's serum-free medium²⁵. *N. brasiliensis* antigen was added at $10 \mu\text{l ml}^{-1}$. The cells were incubated for 5 days as above and the supernatant was collected and filtered. For preparation of the antigen, adult worms were removed from the intestines of infected rats on the eighth day of infection, washed by sedimentation in 0.15 M saline and incubated in Hank's medium at 37 °C for 3 h. The supernatant containing antigenic secretions and excretions of the parasites was adjusted to a protein concentration of 1 mg ml^{-1} and filtered.

medium from antigen-stimulated normal rat mesenteric lymph node cells or from antigen-unstimulated infected or normal lymph node cells does not show these properties. The necessity to stimulate the infected rat cells with antigen to obtain conditioned medium with optimal activity indicates that primed antigen-specific cells must be activated.

Cell separation experiments indicated that the mast cell growth factor is produced by immunoglobulin-negative non-adherent cells. Thus infected rat lymph node cells depleted of plastic-adherent and immunoglobulin-bearing cells yielded a culture fluid of undiminished conditioning activity whereas the

supernatant fluids of cultured adherent or immunoglobulin-bearing cells have little effect. These preliminary experiments suggest, therefore, that mast cell growth factor is produced by antigen-specific T cells contained within the draining lymph nodes of *N. brasiliensis*-infected rats. This conclusion is reinforced by previous observations that the proliferation of mucosal mast cells in helminth infections is dependent on the integrity of the T lymphocyte system of the host⁵⁻⁷ and can be adoptively transferred by immune T cells⁸. Although there has been controversy on this point the evidence now indicates that T cells have an inductive rather than a precursor role in influencing mucosal mast cell numbers⁷.

It is often difficult to distinguish mucosal from connective tissue populations of mast cells as both possess specific receptors for IgE and dense secretory granules containing proteoglycan, histamine and basic proteins. However, mucosal mast cells require special fixation techniques for their detection in tissues and differ from connective tissue mast cells in having granules which are sparser and more variable in size and which contain less strongly sulphated glycosaminoglycans and less monoamine⁹. Additionally, each type of mast cell contains a similar yet antigenically distinct chymotrypsin-like serine protease which can be used to define the type of mast cell present in any preparation^{10,11}.

The mast cells generated in our cultures have the following properties. Morphologically they differ from peritoneal mast cells in being smaller and having sparser granules which show a marked variation in size (Fig. 2). Following fixation of cytocentrifuge preparations in Carnoy's fluid and staining by the astra blue (pH 0.3)/safranin technique, the granules of the cultured mast cells stained exclusively with astra blue whereas, by comparison, those of peritoneal mast cells stained exclusively with safranin. Preparations fixed in Carnoy's fluid and stained with toluidine blue (pH 0.5) were metachromatic. Uptake of IgE by surface receptors of cultured mast cells was demonstrated by overnight incubation with monoclonal rat IgE (IR162) or with serum rich in IgE from *N. brasiliensis* immune rats¹² followed by a 1-h incubation in FITC-labelled purified goat anti-rat Fc ϵ . Finally, extracts of the cultured mast cells contained a very high level of mucosal mast cell protease ($26 \mu\text{g}$ per 10^6 mast cells) (Fig. 3).

Others have observed mast cell proliferation in cultures of various tissues grown in media conditioned by antigen-stimulated^{13,14} or concanavalin A-stimulated lymphocytes¹⁵⁻¹⁷. In these studies the type of mast cell was not ascertained. Ginsburg and colleagues, however, have recently identified two types of mast cells in cultures of mouse lymphoid tissue grown on an embryonic fibroblast monolayer: one type, the proliferation of which was dependent on the presence of antigen-specific T cells or their products, arose from a precursor in the lymphoid cell layer; the other type, not dependent on T-cell factors, arose from cells in the fibroblast layer¹⁸. Although critical discriminating tests were not performed, it was suggested that the lymphoid-derived cells were mucosal mast cells and the feeder layer-derived cells were connective tissue mast cells. As the greatest differentiation and proliferation of 'mucosal' mast cells occurred with mesenteric lymph node cells and few arose from bone marrow cells¹⁹, it was proposed that the 'mucosal' mast cell derived from a precursor in lymphoid tissue¹⁸.

Our present results suggest that the precursor cell for the mucosal mast cells, like the precursor for the connective tissue population²⁰, originates in the bone marrow. The same conclusion has recently been reached independently by Crowle²¹ based on *in vivo* experiments with W/W^o mice. We propose that *in vivo* the precursor cell, after migrating from the bone marrow, would normally differentiate *in situ* in the areas where both the antigen and antigen-specific T cells are to be found—a proposition supported by ultrastructural observations of infected tissues²². It should now be possible to analyse the signals and the steps involved in mast cell proliferation in a system which reflects and is directly relevant to the mastocytosis occurring *in vivo*.

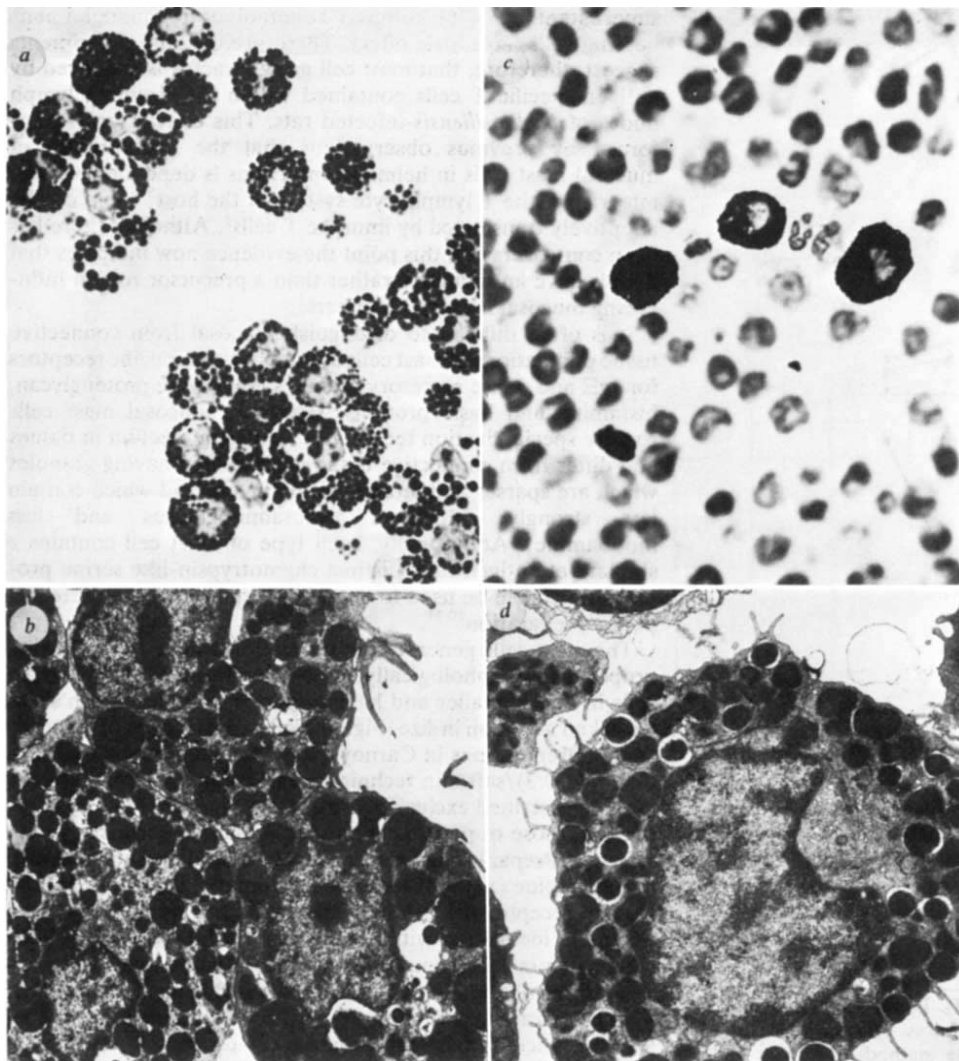


Fig. 2 Morphology of cultured mast cells. For mast cell analysis, bone marrow cells were grown in bulk cultures in 25 cm² tissue culture flasks. Bone marrow was allowed to grow for 2–3 weeks with weekly refeeding and restimulation with 10% fresh conditioned medium. During this time mast cells proliferated to form greater than 90% of the total cell population. Cells in suspension cultured for 21 days were fixed in Karnovsky's aldehyde fixative²⁶ and were pelleted and post-fixed in osmium followed by routine dehydration and Epon embedding. *a*, 1 µm section (×745) stained with methylene blue/azure II. *b*, EM preparation (×3,800) stained in section with uranyl acetate and lead citrate. For comparison, peritoneal exudate cells from syngeneic rats treated in exactly the same way are shown: *c*, 1 µm section (×745) of peritoneal exudate cells. *d*, EM preparation (×5,300) of peritoneal mast cell.

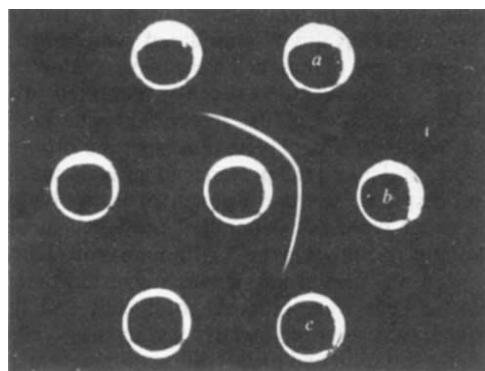


Fig. 3 Double immunodiffusion in 1% agarose to determine the presence of mucosal mast cell protease in extracts of cultured mast cells. Mucosal mast cell protease was purified from small intestine as previously described²⁷. Specific antiserum against mucosal mast cell protease was prepared by injecting rabbits with 50 µg of protease with complete Freund's adjuvant at weekly intervals. Each well contained 30 µl of sample. Centre well, anti-mucosal mast cell protease; *a*, purified protease (150 µg ml⁻¹); *b*, 0.15 M KCl extract (200 µl) of pelleted cells from a culture containing 2 × 10⁶ mast cells; *c*, 0.15 M KCl extract of syngeneic rat peritoneal cells containing 1.9 × 10⁶ mast cells. The results show that the extract of the cultured cells contains a high level of mucosal mast cell protease indicating the presence of mucosal mast cells in the culture. The quantity of mucosal mast cell protease in the cell extract, measured by radial immunodiffusion, was 26 µg per 10⁶ mast cells, representing approximately 13% of the soluble mast cell protein.

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1. Askenase, P. W. *Springer Semin. Immunopath.* **2**, 417–442 (1980).
2. Jarrett, E. E. & Miller, H. R. P. *Prog. Allergy* **31**, 178–233 (1982).
3. Murray, M. in *Immunology to Animal Parasites* (ed. Souby, E. J. L.) 155–190 (Academic, New York, 1972).
4. Mayrhofer, G. *Cell. Immun.* **47**, 304–311 (1979).
5. Ruitenbergh, E. J. & Elgersma, A. *Nature* **264**, 258–260 (1976).
6. Mayrhofer, G. & Fisher, R. *Immunology* **37**, 145–155 (1979).
7. Mayrhofer, G. & Bazin, H. *Int. Archs. Allergy* **64**, 320–331 (1981).
8. Nawa, Y. & Miller, H. R. P. *Cell. Immun.* **42**, 225–239 (1979).
9. Enerback, L. *Monogr. Allergy* **17**, 222–232 (1981).
10. Woodbury, R. G., Gruzinski, G. M. & Lagunoff, D. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2785–2789 (1978).
11. Woodbury, R. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 5311–5313 (1978).
12. Jarrett, E. E. & Bazin, H. *Nature* **251**, 613–614 (1974).
13. Ginsburg, H. & Lagunoff, D. *J. Cell Biol.* **35**, 685–697 (1967).
14. Denburg, J. A., Befus, A. D. & Bienenstock, J. *Immunology* **41**, 195–202 (1980).
15. Schrader, J. W. *J. Immun.* **126**, 452–458 (1981).
16. Nabel, G., Galli, S. J., Dvorak, A. M., Dvorak, H. F. & Cantor, H. *Nature* **291**, 332–334 (1981).
17. Tertian, G., Yung, Y.-P., Guy-Grand, D. & Moore, M. A. S. *J. Immun.* **127**, 788–793 (1981).
18. Ginsburg, H., Ben-Shahar, D. & Ben-David, E. *Immunology* **45**, 371–380 (1982).
19. Ginsburg, H., Olson, E. C., Huff, T. F., Okudaira, H. & Ishizaka, T. *Int. Archs. Allergy* **66**, 447–458 (1981).
20. Kitamura, Y., Yokoyama, M., Matsuda, H., Ohno, T. & Mori, K. *J. Nature* **291**, 159–160 (1981).
21. Crowley, P. K. *Fedn Proc.* **41**, 581 (1982).
22. Miller, H. R. P. *Lab. Invest.* **24**, 339–347 (1971).
23. Sumner, M. A. *et al. Br. J. Haemat.* **23**, 221–234 (1972).
24. Jennings, F. W., Mulligan, W. & Urquhart, G. M. *Exptl Parasit.* **13**, 367–373 (1963).
25. Iscove, N. N. & Melchers, F. *J. exp. Med.* **147**, 923–933 (1978).
26. Karnovsky, M. J. *J. Cell Biol.* **27**, 137A–138A (1965).
27. Katunuma, N. *et al. Eur. J. Biochem.* **52**, 37–50 (1975).

The role of position in determining homoeotic gene function in *Drosophila*

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Homoeotic mutations of *Drosophila* lead to the replacement of one structure by another, for example, *Antennapedia*¹ replaces the antenna with a mesothoracic leg and *bithorax* produces an anterior wing instead of the anterior haltere. The transformed structures differentiated by the homoeotic mutants are essentially normal—only the position in which they appear is abnormal. The mutant phenotypes suggest that in normal development homoeotic genes are involved in developmental alternatives and contribute to a genetic address that defines the particular developmental pathway taken by a primordial group of cells²⁻⁵. Thus, in the absence of homoeotic gene function, primordia in different positions should follow the same basic developmental pathway. This is indeed the case for embryos that show no activity of *bithorax* genes; thoracic and abdominal segments develop as mesothorax⁶. The simplest view on homoeotic gene function is that the genes act selectively on primordia depending on their position in the embryo. To test this hypothesis, we used a mutation at the *Antennapedia* locus, *Antp*^{73b}, which transforms the antenna into a mesothoracic leg, and we observed the function of the homoeotic genes *engrailed* and *Ultrabithorax* in two apparently morphologically identical appendages which develop from primordia in different positions. Our results indicate that position is the relevant factor in the function of these two homoeotic genes.

We have considered the antennal primordium which normally gives rise to the eye, head and antenna of the fly, and the ventral mesothoracic primordium which produces the second leg. Two differences have been described for these two primordia with regard to the function of *engrailed* and *Ultrabithorax*: (1) the subdivision of the original primordium into anterior and posterior polyclones occurs at about 72 h of development (mid-larval period^{7,8}) in the antennal cells, whereas it occurs at about 3 h (blastoderm period) in the mesothoracic leg⁹. As the function of the *engrailed* gene is required for maintenance of the antero-posterior boundary¹⁰⁻¹², this indicates that *engrailed* is active in the mesothorax much earlier than in the antennal segment. (2) Mutations at the *Ultrabithorax* locus (*Ubx*) produce an homoeotic transformation of the posterior mesothoracic leg compartment into the homologous prothoracic one^{13,14}. These mutations have no effect on antennal development, which indicates that the *Ultrabithorax* gene is active in the leg but not in the antenna.

There are two sets of legs of mesothoracic type in flies heterozygous for the dominant mutation *Antp*^{73b}: one in the normal position of the second leg (henceforth referred to as the thoracic leg) and the other in the position of the antenna (to be referred to as the cephalic leg). The bristle pattern of the cephalic leg is typically mesothoracic (Fig. 1). We have studied the period of function of *engrailed* by timing the establishment of the antero-posterior boundary in the cephalic and thoracic legs and the respective requirements for *Ultrabithorax* activity.

In these experiments we induced clones that were marked genetically (for details see Table 1 legend) in different developmental stages to determine the time at which the anterior (A) and posterior (P) compartments of the leg are established. Before this time, individual clones can extend to both compartments but thereafter they cannot (see Table 1). In the thoracic leg, A and P polyclones are segregated after 4 ± 2 h (blastoderm period) which agrees with the results obtained in non-

Antennapedia flies⁹. By contrast, clones in the cephalic leg can extend to both compartments until the mid-larval period (84 ± 12 h after oviposition, *Minute* time). This is about the time when the antero-posterior subdivision occurs in the normal antenna⁸.

We compared the effects of the lack of *Ubx* activity in the cephalic and thoracic legs of *Antennapedia* flies. It has been found recently^{13,14} that the loss of *Ubx*⁺ function in early embryogenesis (blastoderm stage) means that the posterior compartments of mesothoracic and metathoracic legs develop as the posterior compartment of the prothoracic one. The loss of *Ubx*⁺ function at later times does not affect the mesothoracic leg; the metathoracic leg, however, is then transformed into a mesothoracic one. Thus, there is an early function of the *Ubx* gene necessary for the normal development of meso- and metathoracic legs and also a later function necessary for metathoracic development.

We have generated, by X-ray-induced mitotic recombination, *Ubx* clones in *Antennapedia* flies. Genetic mosaics must be used as *Ubx* mutants are zygotic lethal. Most of the *Ubx*⁻ clones were induced at the blastoderm stage although some were also induced during the larval period as it was conceivable that the cephalic leg might show a temporal succession of mutant phenotypes as in the case of the metathoracic leg¹³. Appropriate genetic combinations were constructed (details are given in

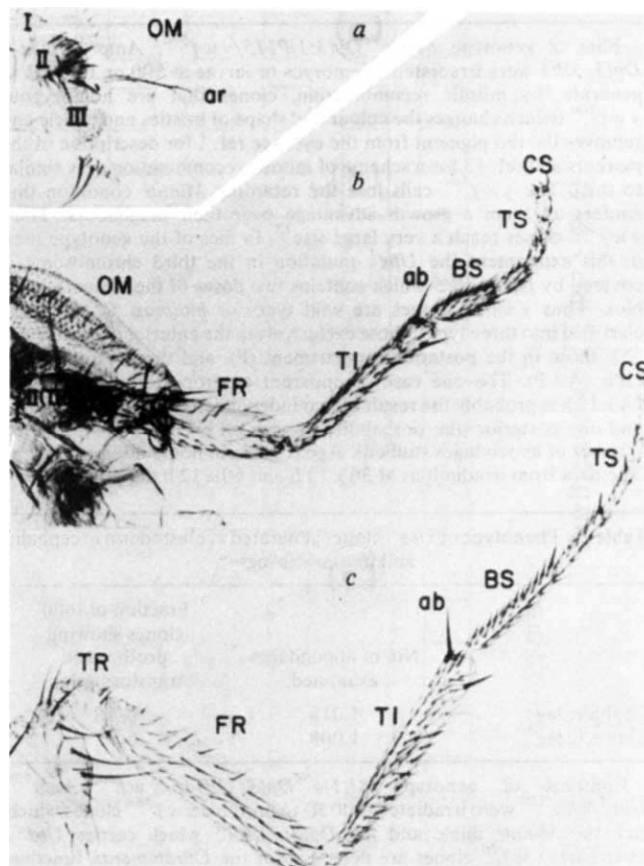


Fig. 1 The cephalic (b) and the second (c) legs of *Antp*^{73b} flies showing the close similarity between them. A normal antenna (a) is included for comparison. The three appendages are shown at the same magnification. The three antennal segments (I, II and III) and arista (ar) are indicated. The apical bristle (ab), typical of the mesothoracic pattern is present in both legs whereas landmarks of first and third leg patterns, such as anterior and posterior transverse rows, are absent. In general, the first antennal segment remains untransformed and the second is modified towards the trochanter. The transformation into leg of mesothoracic type is usually complete distal from the trochanter, although sometimes arista structures remain in the tarsal region. TR, trochanter; FR, femur; TI, tibia; BS, basitarsus; TS, tarsal segments; CS, claws; OM, ommatidia.

Table 2 legend) to allow the detection of *Ubx*⁻ clones. We found that in the thoracic (second) leg, most *Ubx*⁻ clones induced at blastoderm showed the prothoracic transformation in the posterior compartment (Table 2). However, clones of the same genotype and initiated at the same time, failed to show prothoracic transformation in the cephalic leg and instead formed a mesothoracic pattern. In both legs, clones produced during the larval period remained mesothoracic. Thus we conclude that the thoracic leg requires *Ubx*⁺ activity in early development but the cephalic one does not.

The results presented here demonstrate the importance of position in the function of homoeotic genes. In *Antennapedia* flies the antennal primordium, although it develops into a

Table 1 Establishment of the antero-posterior segregation in cephalic and thoracic legs

Age at clone initiation	No. of clones found in anterior and posterior compartments							
	Cephalic leg				Thoracic leg			
	n	A	P	A+P	n	A	P	A+P
Blastoderm (4±2 h)	1,202	12	9	15	1,330	26	10	0
36±12 h	832	17	14	10	—	—	—	—
60±12 h	394	17	9	1	—	—	—	—
84±12 h								

Flies of genotype *M(1)os^{Sp}Dp(3;1)P115/y w f^{36a}; Antp^{73b} Ubx¹/Dp(3;3)P5* were irradiated as embryos or larvae at 500 or 1,000 R to generate, by mitotic recombination, clones that are homozygous *y w f^{36a}* (which changes the colour and shape of bristles and cuticle and removes the red pigment from the eye; see ref. 1 for description of the markers and ref. 13 for a scheme of mitotic recombination very similar to this). The *y w f^{36a}* cells lose the retarding *Minute* condition that confers on them a growth advantage over their neighbours. Thus, *y w f^{36a}* clones reach a very large size¹⁵. In flies of the genotype used in this experiment the *Ubx*¹ mutation in the third chromosome is covered by *Dp(3;3)P5* which contains two doses of the *bithorax* complex. Thus *y w f^{36a}* clones are wild type for *bithorax*. Clones were classified into three types: those exclusively in the anterior compartment (A), those in the posterior compartment (P), and those extending to both (A+P). The one case of apparent anteroposterior crossing at 84±12 h is probably the result of two independent clones, one anterior and one posterior (the probability of such an event was 0.45 for the number of appendages studied). Age is given in hours after egg laying. The data from irradiations at 36±12 h and 60±12 h are pooled.

Table 2 Phenotype of *Ubx*⁻ clones generated at blastoderm in cephalic and in thoracic legs

	No. of appendages examined	Fraction of total clones showing prothoracic transformation
Cephalic leg	1,018	0/18
Thoracic leg	1,008	6/9

Embryos of genotype *M(1)os^{Sp}Dp(3;1)P115/y w f^{36a}; Antp^{73b} Ubx¹/Ubx¹³⁰* were irradiated (500 R) to produce *y w f^{36a}* clones which lack the *Minute* allele and the *Dp(3;1)P115* which carries *Ubx*⁺. Therefore, *y w f^{36a}* clones are defective for the *Ultrabithorax* function and because of the *Minute* effect¹⁵ reach a very large size which permits an unequivocal identification of the bristle pattern as prothoracic or mesothoracic. Clones producing prothoracic transformation are represented as a fraction of the total number of clones found in the posterior cephalic and second thoracic legs. We also found *y w f^{36a}* clones in the third posterior thoracic leg (metathoracic) which showed (8/10) prothoracic transformation. The reason why not all clones in the thoracic legs showed prothoracic transformation is purely technical. In the recombination system used there are always several *y w f^{36a}* clones which arise after mitotic recombination distal to *Dp(3;3)P115* and hence retain the *Ubx*⁺ gene. These have been estimated to represent 20% of the total *y w f^{36a}* clones^{13,14}. Clones generated after the blastoderm period do not show prothoracic transformation in either the cephalic (0/34) or thoracic (0/10) legs, in agreement with previous results^{13,14}.

mesothoracic leg, retains the pattern of homoeotic gene function (at least for *en* and *Ubx*) characteristic of the normal antenna. The thoracic legs in *Antennapedia* flies develop normally with respect to *en* and *Ubx*. The critical factor for the activity of these genes seems to be the position occupied by the primordial cells, not the identity of the structure into which they develop. This conclusion, however, is based on the assumption that there is no difference other than position between the cephalic and thoracic primordia of *Antennapedia* flies. This assumption—although very reasonable, as both primordia develop into the same structure—cannot be rigorously proven at present.

The lack of activity of *Ubx* in the cephalic leg poses an intriguing question about the development of the legs; if the normal development of a leg of mesothoracic type requires (at least in the posterior compartment) a decision prothorax-mesothorax¹³ which is implemented genetically through one of the functions—postprothorax—controlled by the *Ultrabithorax* gene, then one would expect any leg of mesothoracic type to undergo the same decision. But in the case of the cephalic leg of *Antennapedia* that gene function is apparently not required, suggesting that either cephalic and thoracic leg primordia in *Antennapedia* produce the same structure via different sets of decisions or the postprothorax function is provided in the head by another developmentally homologous gene that is inactive in the thorax.

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- Lindsley, D. L. & Grell, E. H. *Carnegie Inst. Wash. Publ.* 627 (1968).
- Lewis, E. B. *Am. Zool.* 3, 33–56 (1963).
- Lewis, E. B. in *The Role of Chromosomes in Development* (ed. Locke, M.) 231–252 (Academic, New York, 1964).
- Morata, G. & Lawrence, P. A. *Nature* 265, 211–216 (1977).
- García-Bellido, A. *Ciba Fdn Symp.* 29, 161–182 (1975).
- Lewis, E. B. *Nature* 276, 565–570 (1978).
- Morata, G. & Lawrence, P. A. *Nature* 274, 473–474 (1978).
- Morata, G. & Lawrence, P. A. *Dev. Biol.* 70, 355–371 (1979).
- Steiner, E. *Wilhelm Roux's Arch. dev. Biol.* 180, 31–46 (1976).
- Morata, G. & Lawrence, P. A. *Nature* 255, 614–617 (1975).
- Lawrence, P. A. & Morata, G. *Dev. Biol.* 50, 321–337 (1976).
- Kornberg, T. *Dev. Biol.* 86, 363–372 (1981).
- Morata, G. & Kerridge, S. *Nature* 290, 778–781 (1981).
- Kerridge, S. & Morata, G. *J. Embryol. exp. Morph.* 68, 211–234 (1982).
- Morata, G. & Ripoll, P. *Dev. Biol.* 42, 211–221 (1975).

Epidermal growth factor rapidly stimulates prolactin gene transcription

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Epidermal growth factor (EGF) was originally characterized as a growth factor for various cell types^{1,2} and was subsequently shown to affect a number of cellular and molecular processes^{3,4}, of which many might be considered as a part of the pleiotropic growth response (enhanced uptake of glucose, uridine and amino acids and stimulated synthesis of protein, RNA and DNA). Very early responses to EGF, such as increased sodium fluxes⁵ and stimulation of tyrosine phosphorylation⁶, have been proposed to mediate some or all of EGF's effects. In a number of tissues, EGF has been shown to increase the synthesis of specific proteins^{7–10}. We have investigated the effects of EGF on prolactin synthesis in the GH₄ rat pituitary cell line to gain further insight into the mechanism of EGF's actions on cellular

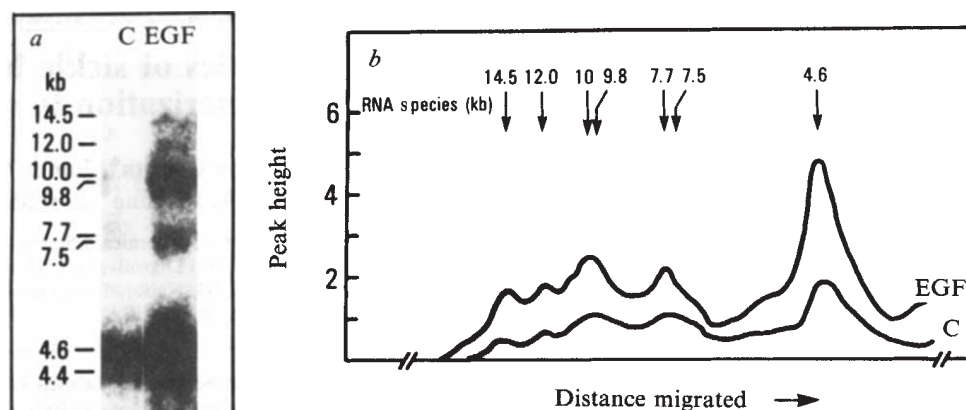
functions. Addition of EGF to GH₄ cells results in a three- to sixfold stimulation of prolactin synthesis, as well as a partial inhibition of cell growth^{7,8}. In this report, we demonstrate that the increased prolactin synthesis appears to be the result of a rapid stimulation of prolactin gene transcription by EGF. It is tempting to speculate that very early transcriptional effects on specific genes, such as reported here in the case of the prolactin gene in GH₄ cells, may mediate some or all of the later effects of EGF on cell cycle regulation in those cells for which it serves as a growth factor.

The synthesis of prolactin in GH₄ cells is regulated by a wide variety of peptide and steroid hormones¹¹⁻¹³. Thyrotropin releasing hormone (TRH) stimulates prolactin synthesis in GH₄ cells by acting at the nuclear level to increase the production of prolactin mRNA¹⁴. The increased steady state content of the primary transcript of the prolactin gene suggested that a transcriptional mechanism was involved. In order to determine whether EGF was also acting at a transcriptional level to increase prolactin mRNA, total cellular poly(A) RNA from control and EGF treated cells was analysed using RNA blotting procedures after size fractionation on denaturing agarose gels¹⁵.

Figure 1a shows that EGF promotes an increase in the steady state level of several nuclear prolactin RNA species including the primary transcript. Visualization of the nuclear species is enhanced by hybridizing the blotted RNA with both labelled cloned prolactin gene-specific intervening sequence and prolactin cDNA. Figure 1b shows a densitometric scan of the larger prolactin nuclear transcripts. In this experiment, there is approximately a fourfold stimulation of the steady state level of each of the prolactin putative nuclear precursors, including the primary transcript. This is comparable to the three- to sixfold EGF stimulation of mature prolactin mRNA levels which are typically observed.

Because of their presumed very short half life, the steady-state level of prolactin nuclear transcripts has been proposed to accurately reflect prolactin gene transcription¹⁴. To verify this, the time course of EGF stimulation of prolactin gene transcription was measured by elongating nascent prolactin RNA transcripts in isolated nuclei as well as by quantitating nuclear precursors by Northern gel analysis. The time course of EGF stimulation of prolactin gene transcription, as well as the resultant cytoplasmic prolactin mRNA accumulation is shown in Fig. 2. The close agreement between the two measurements of transcription confirms previous predictions that changes in nuclear RNA precursor levels can represent an accurate estimation of the transcriptional activity of specific genes.

Fig. 1 Effects of EGF on the level of nuclear prolactin RNAs. *a*, Poly(A) RNA was prepared from control GH₄ cells (C) and from cells treated with 5×10^8 M EGF for 24 h (EGF) as previously described¹⁴. Aliquots of 12 μ g of each RNA were denatured by heating (65 °C) in sample buffer (10 mM NaH₂PO₄, pH 7.4, 2.2 M formaldehyde, 50% (v/v) formamide). After electrophoresis on a 1.5% agarose gel containing 1.1 M formaldehyde, the RNA was transferred to 'diazotised' paper (DBM). The RNA was hybridized with 10^7 c.p.m. per ml of both nick-translated prolactin cDNA and prolactin gene specific intervening sequence, pIVSc (specific activity $3-8 \times 10^8$ c.p.m. per μ g with [α -³²P]dCTP) for 16 h at 42 °C. The autoradiogram was exposed for 2 days to clearly visualize the larger nuclear prolactin RNAs. The lower portion of the gel containing mature prolactin mRNA was drastically overexposed and is not shown. The sizes of the prolactin nuclear RNAs are extrapolated from the migration of 18S and 28S RNA. The largest (14.5 kb) species (alternatively determined to be 12.5 kb when DNA standards are used, data not shown) appears to be the true size of the gene (S. Supowit *et al.*, in preparation) and is therefore referred to as the primary transcript. *b*, Densitometric scan of the autoradiograph shown in *a*. Stimulation of each nuclear prolactin RNA (based upon comparison of peak areas) is approximately 3.8-fold.



EGF maximally stimulates prolactin gene transcription 10-fold within 1 h, from 2 to 18 p.p.m. per kilobase, using a 0.9 kilobase prolactin genomic intervening sequence probe. This stimulation is followed by a rapid attenuation, such that 3 h following EGF addition prolactin gene transcription is only half of its maximally induced level. The net effect of this burst of prolactin transcription is to rapidly elevate the cytoplasmic prolactin mRNA content. There is a further slower transcriptional attenuation occurring over a period of days and during this time the prolactin mRNA levels remain fairly constant, paralleling the slow decline in transcription. In some cases the attenuation event has been more pronounced such that transcription returns to control levels within the first 36 h, while in others it remains moderately elevated for days. Neither the initial rapid nor subsequent slower attenuation can be accounted for by reciprocal changes in total polymerase II transcriptional activity.

The data summarized in Fig. 2 demonstrate that the kinetics of prolactin mRNA accumulation in response to EGF reflect the kinetics of effects on prolactin gene transcription. Since a steady-state transcriptional effect is not achieved, the kinetics of EGF-stimulated prolactin mRNA accumulation is not a reflection of its cytoplasmic half life. Thus, the accumulation of prolactin mRNA ceases 10 h after the addition of EGF because the increased rate of production of newly transcribed prolactin mRNA sequences no longer exceeds the rate of cytoplasmic decay. When prolactin mRNA stability is estimated under conditions of steady-state transcription, the half-life is approximately 6 h (ref. 16). Following prolonged incubations with EGF, there is an increase in cytoplasmic prolactin mRNA without a concomitant increase in transcription (Fig. 2). The mechanism of this late effect of EGF is the subject of current investigation.

The mechanism of the EGF induced transcription is unclear. There are a variety of second messenger systems which have been associated with EGF. A large body of evidence suggests that the EGF receptor is a tyrosine kinase which is capable of autophosphorylation in addition to the phosphorylation of other membrane proteins^{6,17,18}. Various cytoplasmic and cytoskeletal proteins may also be substrates, either directly or indirectly¹⁹. However, as yet, no nuclear protein has been shown to be modified by tyrosine phosphorylation in response to EGF treatment. EGF stimulation of sodium fluxes has also been suggested as a critical intermediate in the regulation of cellular processes by EGF⁵. Increased cytoplasmic sodium can affect the homeostasis of other ions including calcium²⁰, which has been suggested to play a role in regulating prolactin gene expression²¹.

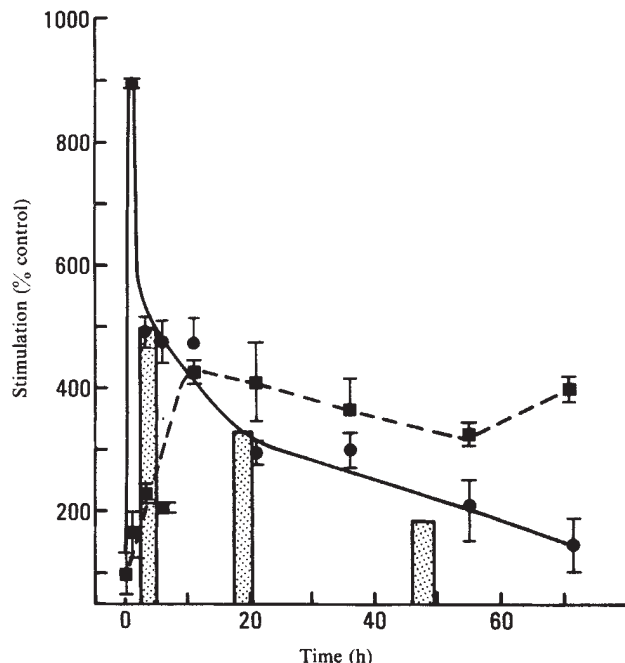


Fig. 2 Time course of EGF stimulation of prolactin gene transcription rate (●), prolactin precursor RNA level (stippled bars) and cytoplasmic prolactin mRNA (○). GH₄ cells were incubated with 5×10^{-8} M EGF for the indicated times before being collected by scraping into iced phosphate-buffered saline (PBS). After washing in PBS, cells were lysed by gentle homogenization in 150 mM KCl, 5 mM MgCl₂, 5 mM DTT, 10 mM Tris pH 7.8, 0.1% Nonidet P-40. After low-speed centrifugation, total RNA was prepared from the cytoplasm by phenol/chloroform extraction. Aliquots of 20 μ g were bound to 1 cm squares of activated DBM paper and hybridized to excess nick-translated cloned prolactin cDNA to quantify prolactin mRNA content. Each point represents the mean $\pm$ s.e. of triplicate determinations. The nuclear pellets were washed in the nascent chain labelling buffer (150 mM KCl, 5 mM MgCl₂, 1 mM MnCl₂, 10 mM Tris, pH 7.8, 10% glycerol). Nuclei were then incubated (25 °C, 45 min) in the same buffer supplemented with 1 mM ATP, CTP and GTP and 200 μ Ci [³²P]UTP (200–400 Ci mM⁻¹) allowing nascent transcripts to elongate. Labelled RNA was purified and quantified as described in refs 24 and 25. Briefly, 8–15 $\times 10^6$ c.p.m. of ³²P-labelled RNA was hybridized to 2 μ g of a 0.9 kb subcloned fragment of a prolactin gene specific intervening sequence (pIVSc) bound to a 3 mm disk of nitrocellulose. Each point is the mean $\pm$ s.e. of the transcription rate (specific c.p.m. bound/c.p.m. added) based upon triplicate hybridizations. Both control and EGF stimulated prolactin gene transcription is undetectable when α -amanitin (0.8 μ g ml⁻¹) is included in the incubation. The hybridization is linear with added labelled RNA and constant with variable DNA/filter (0.5–5.0 μ g). The use of a cloned intervening sequence fragment during the hybridization prevents variable competition by accumulated mRNA. Total cellular poly(A) containing RNA was prepared from cells treated in parallel. Prolactin RNA precursors were quantified as described in Fig. 1. For comparison purposes all data are expressed as percent control. Basal prolactin gene transcription rate in GH₄ cells is 2 p.p.m.; maximal stimulation is 18 p.p.m. Basal prolactin mRNA level is 1,200 molecules per cell; maximal stimulation is 5,300 molecules per cell.

Alternatively, it is possible that a direct interaction of rapidly internalized EGF–receptor complexes with the nucleus mediates the transcriptional effects. However, the shape of the time course of prolactin transcriptional stimulation, a burst followed by rapid attenuation, is consistent with the time course of the binding of EGF to surface receptors in cultured cells, a transient maximum followed by rapid internalization²². This time course is distinct from the gradual constant accumulation of nuclear membrane bound EGF²³. These data suggest that the putative nuclear binding events are not involved with the transcriptional regulation of prolactin.

An increase in prolactin gene transcription rate appears to be the major, if not sole, mechanism for the EGF stimulation of prolactin synthesis in GH₄ cells. The burst-attenuation kinetics of the transcriptional stimulation result in the rapid elevation of the cytoplasmic prolactin mRNA to the maximal levels. This result demonstrates that caution should be exercised

in interpreting half times of accumulation as a reflection of message stability. We suggest that burst-attenuation transcriptional kinetics could be an important physiological mechanism for rapidly increasing the cytoplasmic content of specific long-lived mRNAs to new steady-state levels in response to external stimuli. The stimulation of prolactin transcription by EGF is too rapid to be a secondary consequence of any effect on the cell cycle and kinetic analysis suggests that the transcriptional effects are closely linked to the interaction of EGF with its receptor on the surface of the cell. It is likely, therefore, that regulation of prolactin gene transcription is mediated by a process that is common to the actions of EGF in other target tissues including those for which it is a positive growth factor. Transcriptional regulation of a specific protein(s) is, therefore, proposed to be among the 'early' events produced by growth factors, and may mediate the observed regulation of the cell cycle by growth factors.

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1. Savage, C. R. & Cohen, S. *J. biol. Chem.* **247**, 7609–7611 (1972).
2. Hollenberg, M. D. & Cuatrecasas, P. *Proc. natn. Acad. Sci. U.S.A.* **70**, 2964–2968 (1973).
3. Carpenter, G. & Cohen, S. *Rev. Biochem.* **48**, 193–216 (1979).
4. Rozengurt, E. *Curr. Topics cell. Regulation* **17**, 59–88 (1980).
5. Rozengurt, E. & Heppell, L. A. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4492–4495 (1975).
6. Ushiro, H. & Cohen, S. *J. biol. Chem.* **255**, 8363–8365 (1980).
7. Schonbrunn, A., Krasnoff, M., Westendorf, J. M. & Tashjian, A. H. Jr *J. Cell Biol.* **85**, 786–797 (1980).
8. Johnson, L. K., Baxter, J. D., Vladavsky, I. & Gospodarowicz, D. *Proc. natn. Acad. Sci. U.S.A.* **77**, 394–398 (1980).
9. Chen, L. B., Gudor, R. C., Sun, T. T., Chen, A. B. & Mosesson, M. W. *Science* **197**, 776–778 (1977).
10. Benveniste, R. et al. *J. clin. Endocr. Metab.* **46**, 169–172 (1978).
11. Ivarie, R. D., Morris, J. A. & Eberhardt, N. L. *Recent Prog. Horm. Res.* **36**, 195–239 (1980).
12. Tashjian, A. H. Jr *Meth. Enzym.* **58**, 527–535 (1979).
13. Bancroft, F. C. in *Functionally Differentiated Cell Lines* (ed. Sato, x. x.) 47–59 (Liss, New York, 1980).
14. Potter, E., Nicolaisen, A. K., Ong, E. S., Evans, R. M. & Rosenfeld, M. G. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6662–6666 (1981).
15. Alwine, J. C., Kemp, D. J. & Stark, G. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5350–5354 (1977).
16. Murdoch, G. H., Rosenfeld, M. G. & Evans, R. M. *Science* (submitted).
17. Carpenter, C., King, L. & Cohen, S. *Nature* **276**, 409–410 (1978).
18. Cohen, S., Carpenter, G. & King, L. *J. biol. Chem.* **255**, 4834–4842 (1980).
19. Hunter, T. & Cooper, J. A. *Cell* **24**, 741–752 (1981).
20. Rozengurt, E. & Mendoza, S. *Ann. N.Y. Acad. Sci.* **339**, 132–138 (1980).
21. White, B. A., Bauerle, L. R. & Bancroft, F. C. *J. biol. Chem.* **256**, 5942–5945 (1981).
22. Haigler, H. T., Maxfield, F. R., Willingham, M. C. & Pastan, I. *J. biol. Chem.* **255**, 1239–1241 (1980).
23. Johnson, L. K., Vladavsky, I., Baxter, J. D. & Gospodarowicz, D. *Nature* **287**, 340–343 (1980).
24. McKnight, G. S. & Palmiter, R. D. *J. biol. Chem.* **254**, 9050–9058 (1979).
25. Compere, S. J., McKnight, G. S. & Palmiter, R. D. *J. biol. Chem.* **256**, 6341–6347 (1981).

Kinetics of sickle haemoglobin polymerization in single red cells

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Kinetic studies on solutions of purified haemoglobin S indicate that the rate of intracellular polymerization is an important variable in the pathophysiology of sickle cell disease^{1–4}. Until now, however, no experimental technique has been available to measure directly the kinetics of intracellular polymerization. Indirect methods, which use visual determination of cellular shape changes or changes in filterability of red cell suspensions, have given apparently conflicting results^{5–9}. Here we report our initial results on the application of a laser-photolysis, light scattering technique to measure directly the kinetics of haemo-

globin S polymerization in single red cells. In our experiment, deoxyhaemoglobin S is rapidly formed by photolysing the carbon monoxide complex with an argon ion laser focused inside the cell, and the change in scattered light is used to detect the appearance of polymer. We find a very wide distribution of delay times, ranging from 1 ms to >100 s, indicating that the polymerization inside red cells proceeds by the same nucleation and growth mechanism as in solutions of purified haemoglobin S¹⁰.

Red cells from patients with homozygous sickle cell disease were washed and diluted to a volume fraction of about 0.001 in a buffer consisting of 0.12 M sodium chloride, 2.4 mM potassium chloride, 15 mM disodium hydrogen phosphate, 2.5 mM sodium dihydrogen phosphate, 5.5 mM dextrose and 1 g l⁻¹ bovine serum albumin. The oxygen in the cell suspension was exchanged for carbon monoxide, and sodium dithionite was then added anaerobically to a final concentration of 10 mM to remove any remaining traces of oxygen. The final room temperature pH was 7.4, and the osmolality determined from the freezing point depression was 290–300 mOsmol. Cells were sedimented onto the top coverslip of a thermostated Dvorak-Stottler flow cell¹¹. An argon ion laser at 514 nm was focused inside a single red cell to a diameter of 3 μ m (full width at half peak intensity) with a thermostated 90 $\times$ oil immersion objective having a numerical aperture of 1.32. The laser was directed to the objective via a half-silvered mirror mounted above it at 45°. Light reflected and scattered by the red cell was collected by the objective, focused with an eyepiece onto a pinhole with a diameter of the magnified image of the cell, and projected onto a photomultiplier tube with a second eyepiece, using a Leitz MPS photometer attachment. The directly reflected laser beam was blocked by masking the centre of the tube lens mounted above the half-silvered mirror. Laser heating of the red cell was determined in experiments on cresol red to be less than 1 °C at the centre of the beam using a peak power density of $\sim 1,000$ W cm⁻² to achieve full photolysis.

To investigate the kinetics of intracellular polymerization at 37 °C systematically, progress curves were recorded for 453 different cells from four patients with homozygous sickle cell (SS) disease. Cells were never kept at 37 °C for more than 3 h, as previous experiments had indicated that changes in the kinetics occurred after this time. Cells were chosen at random and no results were excluded from the data analysis.

Figure 1 shows representative progress curves for sickle cells, for normal cells and for solutions of purified haemoglobin S. Normal cells gave a noisy signal which did not change with time. A variety of progress curves were observed for sickle cells. These could be roughly divided into two types. The first type resulted from the most rapid intracellular polymerization, and was very similar to that found for polymerization from solution. There was a clear delay followed by a sudden increase in light scattering, where the average increase in the scattered intensity was the same as that found for solution polymerization. A significant difference between the solution and cell progress curves was that the increase in light scattering was accompanied by a marked decrease in the amplitude of the noise (Fig. 1). The amplitude of the noise during the delay period was much greater than that found for solution, and was very similar to the noise observed for normal cells (Fig. 1). The noise from normal cells and from sickle cells during the delay period probably corresponds to the previously described 'flicker phenomenon', which is thought to arise from brownian motion^{11–14}. Fluctuations in cell thickness from brownian motion could produce fluctuations in the measured intensity by modulating the interference between laser light reflected from the top and bottom cell surfaces. The increase in internal viscosity resulting from polymer formation would then decrease the noise by damping the thickness fluctuations.

The second type of progress curve was found for cells exhibiting apparently slow intracellular polymerization. These curves showed a sudden and pronounced decrease in the amplitude of the noise, but the change in average light scattering following the noise decrease varied enormously from cell to cell, and in repeated measurements on the same cell. We interpret these distorted kinetic progress curves as resulting from cell motion that is occurring simultaneously with polymer formation, that is, morphological 'sickling'^{5–9}. For these cells the delay time was taken as the time of the decrease in noise. Visual observations showed that there was no morphological change in the cell during the delay period measured in this way.

For delay times less than 0.03 s almost all cells exhibited polymerization type progress curves, while for cells with delay times longer than about 3 s there was no increase in light scattering from polymerization following the delay period. Of the 453 cells examined, 276 (61%) exhibited solution type polymerization progress curves, 146 (32%) exhibited progress

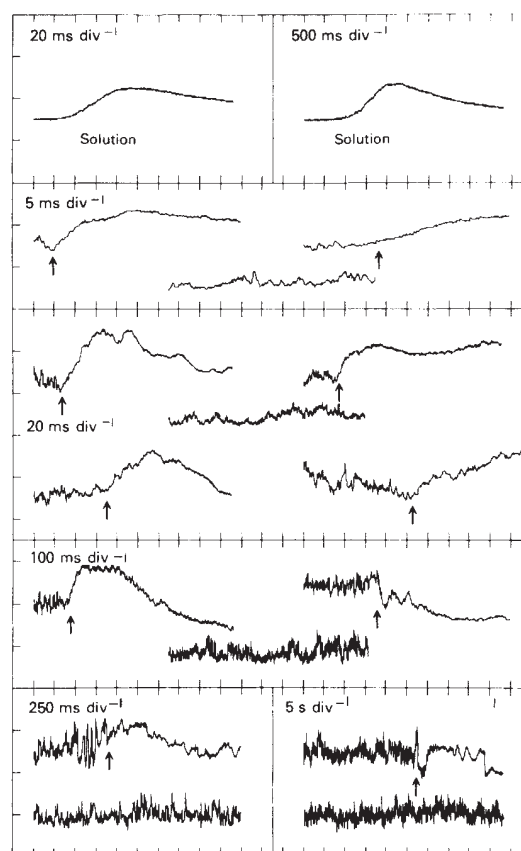


Fig. 1 Representative progress curves for purified haemoglobin S solutions, sickle cells and normal cells. The time scale in milliseconds per division (ms div⁻¹) is given. Each division on the vertical axis is 1 V. No normalization has been applied to the amplitudes. The two progress curves at the top of the figure were measured on a 2.7- μ m layer of a 0.34 g cm⁻³ solution of purified carbonmonoxyhaemoglobin S at 37 °C and on a 1.8- μ m layer of a 0.28 g cm⁻³ solution at room temperature. Both solutions contained 0.15 M potassium phosphate and 0.055 M sodium dithionite, pH 7.1. Progress curves are shown for 10 sickle cells and 5 normal cells. The delay time for the sickle cells is indicated by a vertical arrow. The curves were selected using the following procedure. The curves were arranged in order of increasing delay time and divided into 10 groups of 42 curves each. Curves were chosen as 'representative' if they met the following criteria: (1) the delay time was within half the standard deviation from the average delay time for the group (except for the group containing cells with the longest delay times); (2) the polymerization amplitude (1.0 ± 0.5 V) and the offset (1.8 ± 0.9 V) were within half the standard deviation for the mean of all measurements; (3) the peak-to-peak noise before and after the delay were within 1 s.d. from the mean for the group. 32 out of the 422 curves met these criteria.

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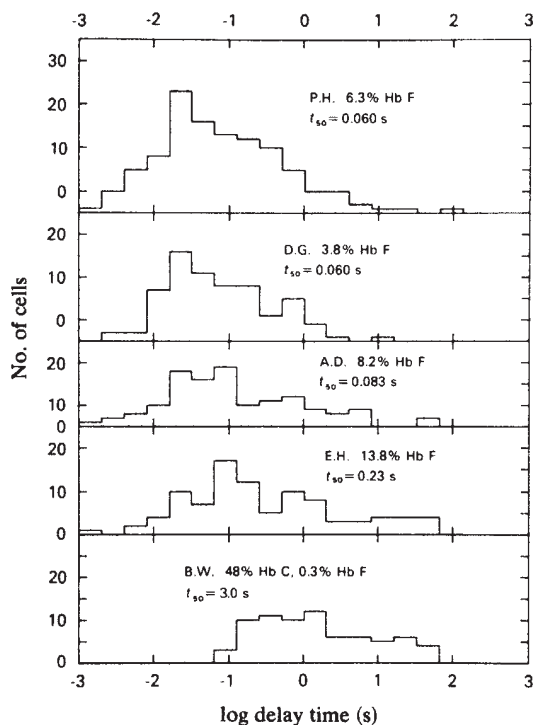


Fig. 2 Histogram of delay times on a logarithmic scale at 37 °C for red cells from four patients with homozygous SS disease and one patient with SC disease. The median delay time (t_{50}) and per cent fetal haemoglobin (Hb F) measured by the alkaline denaturation method are given in the figure. The number of cells with delay times >50–100 s were 4 out of a total of 155 cells for patient P.H., 4 of 105 for D.G., 4 of 87 for A.D., 6 of 101 for E.H., and 21 of 94 for B.W. These cells were included in the calculation of the median delay time.

curves with delay times determined by the noise decrease, 13 (3%) exhibited uninterpretable progress curves and 18 (4%) exhibited progress curves similar to normal cells for 50–100 s, with no morphological evidence of intracellular polymerization at the end of this observation period. Progress curves were not recorded for more than 50–100 s, as this would have significantly slowed data collection.

An interesting difference between cells exhibiting rapid and slow polymerization was found in the reproducibility of the delay time. In a separate series of experiments progress curves were measured repeatedly on 13 cells with mean delay times ranging over 0.05 to 11 s. For the nine cells with mean delay times <3 s the percentage standard deviation from the mean was 19%, with a range of 10–36%. Measurements could be repeated an average of 23 times (range 11–30) before the morphological change produced by intracellular polymerization became irreversible, and further experiments yielded uninterpretable progress curves. For the four cells with mean delay times longer than 3 s, the measurements could be repeated on average only 11 times (range 8–14), and the delay times were much less reproducible, with an average standard deviation of 71% (range 52–99%). This irreproducibility of long delay times is also characteristic of polymerization in solution, and has been previously interpreted as arising from the stochastics of the homogeneous nucleation of single polymers in the small sample volumes ($5 \times 10^{-11} \text{ cm}^3$)¹⁰.

Figure 2 gives the main results of our kinetic study, which shows the delay time histogram for the four patients with homozygous SS disease. There is an extremely wide distribution of delay times, varying from 1 ms to >100 s. This wide range of delay times was expected¹ from the known heterogeneity in intracellular haemoglobin S concentration¹⁵ and the enormous sensitivity of the delay time of solution polymerization to initial haemoglobin S concentration (delay time is inversely proportional to the 15th to 40th power of concentration)^{1,10,16}. Solution studies have shown that other variable cellular factors,

such as pH^{16,17} and 2,3-diphosphoglycerate levels^{18,19}, are relatively unimportant in determining the delay time.

To compare quantitatively the delay times observed in cells and in isolated solutions, we calculated the distribution of intracellular haemoglobin S concentrations from the delay time histogram (Fig. 3). For this calculation we used the sum of the delay time histograms for the three patients with fetal haemoglobin levels below 9% and the concentration dependence of the delay time measured on purified haemoglobin S solutions using a similar laser-photolysis light scattering technique¹⁰. The calculated distribution in Fig. 3 yields a mean value of 0.32 g cm^{-3} for the intracellular haemoglobin S concentration, which is in excellent agreement with the mean value of 0.30 ± 0.02 to $0.32 \pm 0.02 \text{ g cm}^{-3}$ measured by conventional methods^{15,20–22}. The concentration distribution shows that the intracellular haemoglobin S concentration extends to > 0.40 g cm^{-3} , in agreement with measurements on density fractionated cells^{15,23} (M. R. Clark and S. B. Shohet, personal communication). The extension of the calculated distribution to very low values of haemoglobin S concentration presumably results from the cells containing a large fraction of haemoglobin

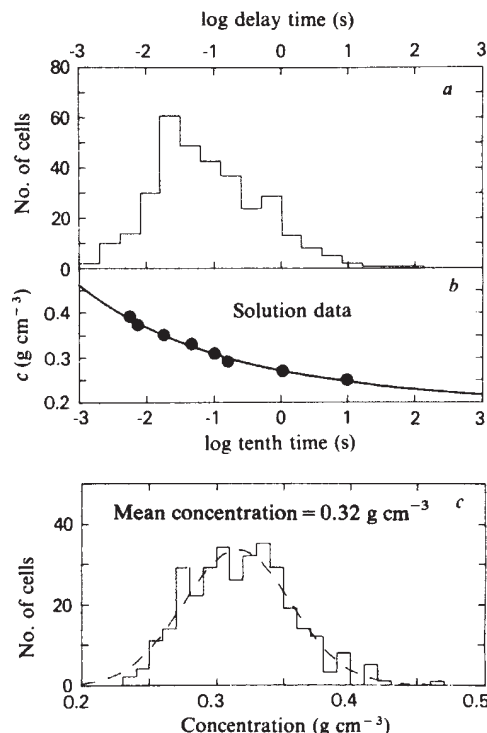


Fig. 3 Determination of the concentration distribution in sickle cells from the delay time distribution. *a*, This histogram of delay times on a logarithmic scale is the sum of the histograms from Fig. 2 for patients P.H., D.G. and A.D. The histogram from patient E.H. was not included, as the fetal haemoglobin content of 13.8% is more than 2 s.d. from the mean of $5.6 \pm 3.6\%$ found for sickle cell patients^{20,21}. *b*, The concentration of purified carbonmonoxyhaemoglobin S is plotted against the logarithm of the tenth-time. The tenth-time is the time required to reach 1/10 of the polymerization amplitude, and is not significantly different from the delay time taken as the time of onset of a measurable increase in light scattering, which was the criterion used for the sickle cell progress curves. The data (filled circles) were obtained by Ferrone *et al.*¹⁰, using the same laser-light scattering photolysis technique for purified haemoglobin S at 35 °C in 0.15 M potassium phosphate buffer, 0.055 M sodium dithionite, pH 7.1. The curve through the data is a least-squares fit of the empirical function $c \text{ (g cm}^{-3}\text{)} = a \exp(-bx + dx^2) + e$, where x is the logarithm of the tenth-time in seconds, $a = 0.104$, $b = 0.287$, $d = 0.021$ and $e = 0.167$. In carrying out the fit the data points were weighted according to the reciprocal of their standard errors. *c*, The histogram of intracellular haemoglobin S concentrations was obtained by calculating the concentration corresponding to each delay time with the empirical function used to draw the curve in *b*. The data are reasonably well represented by a gaussian function (dashed curve), $P = a \exp(-(b-c)/d)^2$, where c is the intracellular haemoglobin S concentration in g cm^{-3} , $a = 33.5$, $b = 0.316$, $d = 0.057$. The detailed shape of the concentration distribution is least certain above 0.40 g cm^{-3} , where there are no solution data.

F^{24} , which is known to increase the delay time markedly^{3,25}. The high fetal haemoglobin content in the cells from patient E.H., moreover, is most probably responsible for the shift of the delay time distribution (Fig. 2) to longer times.

The close correspondence of the calculated and measured concentration distributions, the similarity in shape of the cell and solution polymerization progress curves, and the irreproducibility of the long delay times in cells together argue strongly that polymerization of haemoglobin S inside cells proceeds by the same nucleation and growth mechanism as in purified solutions. The results of a previous kinetic study, which showed that red cell membrane components have no significant effect on the delay time of purified solutions²⁶, considerably strengthen this conclusion.

Delay time measurements on cells should provide a sensitive method for assaying potential therapeutic agents. To evaluate

the results, however, it will first be necessary to establish criteria for effectiveness by measuring delay time distributions for sickle cell syndromes of varying clinical severity. As an example, we have measured the distribution of delay times from a patient with sickle cell haemoglobin C (SC) disease (Fig. 2). The median delay time (t_{50}) is 3 s, much longer than the t_{50} of 0.06–0.23 s for the SS patients (Fig. 2). The delay time distribution found for SC cells is consistent with the clinical finding that SC disease is intermediate in severity between SS disease and the essentially benign sickle cell trait²⁰, in which the delay times are much longer^{7,27}.

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- Hofrichter, J., Ross, P. D. & Eaton, W. A. *Proc. natn. Acad. Sci. U.S.A.* **71**, 4864–4868 (1974).
- Eaton, W. A., Hofrichter, J. & Ross, P. D. *Blood* **47**, 621–627 (1976).
- Sunshine, H. R., Hofrichter, J. & Eaton, W. A. *Nature* **275**, 238–240 (1978).
- Sunshine, H. R., Ferrone, F. A., Hofrichter, J. & Eaton, W. A. *INSERM Symp.* **9**, 31–46 (1979).
- Ramplung, M. W. & Sirs, J. A. *Clin. Sci. molec. Med.* **45**, 655–664 (1973).
- Messer, M. J. & Harris, J. W. *J. Lab. clin. Med.* **76**, 537–547 (1970).
- Karowsky, H. S. & Hochmuth, R. M. *J. clin. Invest.* **56**, 1023–1034 (1975).
- Antonini, E. *et al.* *FEBS Lett.* **86**, 209–212 (1978).
- Harrington, J. P. & Nagel, R. L. *J. Lab. clin. Med.* **90**, 863–872 (1977).
- Ferrone, F. A., Hofrichter, J., Sunshine, H. R. & Eaton, W. A. *Biophys. J.* **32**, 361–377 (1980).
- Dvorak, J. A. & Stotler, W. F. *Expl. Cell. Res.* **68**, 144–148 (1971).
- Parpart, A. K. & Hoffman, J. F. *J. cell. comp. Physiol.* **47**, 295–303 (1956).
- Padilla, F., Bromberg, P. A. & Jensen, W. N. *Blood* **41**, 653–660 (1973).
- Burton, A. L., Anderson, W. L. & Andrews, R. V. *Blood* **32**, 819–822 (1968).

- Seakins, M., Gibbs, W. N., Milner, P. F. & Bertles, J. F. *J. clin. Invest.* **52**, 422–432 (1968).
- Hofrichter, J., Ross, P. D. & Eaton, W. A. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3035–3039 (1976).
- Goldberg, M. A., Husson, M. A. & Bunn, H. F. *J. biol. Chem.* **252**, 3414–3421 (1977).
- Swerdlow, P. H. *et al.* *Hemoglobin* **1**, 527–537 (1977).
- Bookchin, R. M., Ueda, Y., Nagel, R. L. & Landau, L. C. in *Biochemical and Clinical Aspects of Hemoglobin Abnormalities* (ed. Caughey, W. S.) 57–64 (Academic, New York, 1978).
- Serjeant, G. R. *The Clinical Features of Sickle Cell Disease* (North-Holland, Amsterdam, 1974).
- Wrightstone, R. N. & Huisman, T. H. *J. Am. J. clin. Path.* **61**, 375–381 (1974).
- Powars, D. R., Schroeder, W. A., Weiss, J. N., Chan, L. S. & Azen, S. P. *J. clin. Invest.* **65**, 732–740 (1980).
- Fabry, M. E. & Nagel, R. L. *Blood Cells* **8**, 9–15 (1982).
- Dover, G. J., Boyer, S. H., Charache, S. & Heintzelman, K. *New Engl. J. Med.* **299**, 1428–1434 (1978).
- Sunshine, H. R., Hofrichter, J. & Eaton, W. A. *J. molec. Biol.* **133**, 435–467 (1979).
- Goldberg, M. A., Lalos, A. T. & Bunn, H. F. *J. biol. Chem.* **256**, 193–197 (1981).
- Bookchin, R. M., Balazs, T. & Landau, L. C. *J. Lab. clin. Med.* **87**, 597–616 (1976).

Protein dynamics and NMR relaxation: comparison of simulations with experiment

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Comparisons between molecular dynamics simulations of proteins and experiment have been based on temperature factors^{1–5} derived from X-ray spectra and on the stability of hydrogen bonds⁶. Here we present a novel method of testing molecular dynamics simulations against nuclear magnetic resonance (NMR) relaxation measurements, based on the recently developed model-free approach⁷ to the interpretation of NMR data. As NMR relaxation in proteins is determined by dynamics on the picosecond–nanosecond time scale, a comparison of NMR experiments and molecular dynamics simulations that last for less than ~100 ps is more meaningful than previous ones^{3–6} involving much longer time scales. We make contact between a molecular dynamics simulation⁸ and a ¹³C-NMR relaxation study⁹ of pancreatic trypsin inhibitor (PTI) by comparing generalized order parameters (which are measures of the extent of angular motion of the bonds) extracted from the relaxation data⁹ with those calculated¹⁰ from a 96-ps trajectory⁸. We show that the theoretical order parameters indicate less motion than their experimental counterparts. The relative flexibility of the residues studied, however, is reasonably well described by the simulation.

At presently available spectrometer frequencies, ¹³C-NMR relaxation of protonated carbons is determined by the

reorientation of the ¹³C–H bond vectors relative to the external magnetic field. For a protein in solution both the motion of the macromolecule as a whole and internal motions in a macromolecule-fixed frame contribute to the relaxation. It has been shown recently⁷ that the unique information on fast internal motions contained in NMR relaxation experiments can be specified by two model-independent quantities: an effective correlation time, τ_e , and a generalized order parameter, $\mathcal{S}$. These quantities can be extracted from relaxation data using a simple fitting procedure. τ_e is proportional to the area of the time correlation function for internal motions; the time correlation function can have components which decay with correlation times faster or slower than τ_e . For ¹³C-NMR of protonated carbons, $\mathcal{S}$ is a measure of the angular restriction of the motion of a ¹³C–H vector and is defined as

$$\mathcal{S}^2 = \frac{4\pi}{5} \sum_{m=-2}^2 \langle |Y_{2m}(\Omega)|^2 \rangle \quad (1)$$

where Y_{2m} are spherical harmonics, Ω specifies the orientation of a ¹³C–H bond in a macromolecule-fixed frame and the angular brackets denote an average over all possible orientations accessible on a time scale shorter than the correlation time for the overall reorientation of the macromolecule (~4 ns for PTI). It follows from equation (1) that $0 \leq \mathcal{S}^2 \leq 1$ and that $\mathcal{S} = 0$ when the internal motion is isotropic, while $\mathcal{S} = 1$ when the motion is completely restricted.

Values of $\mathcal{S}$ and τ_e of ¹³C–H bonds for 12 methyl groups have been extracted⁷ from the relaxation data of Richarz *et al.*⁹. The τ_e values which contain contributions due to motions of and about the symmetry axis of the methyl groups, were in the range 19–70 ps.

As in the molecular dynamics simulation of Karplus and McCammon⁸ the hydrogens are incorporated into the heavy atoms to which they are bound, it is impossible to calculate $\mathcal{S}$ and τ_e for methyl groups directly from their trajectory. However, a comparison with experimental order parameters becomes possible if it is assumed that the motion of the methyl symmetry axis (for example, the C α –C β bond in alanine) and the motion of the protons about this axis are uncoupled. In this

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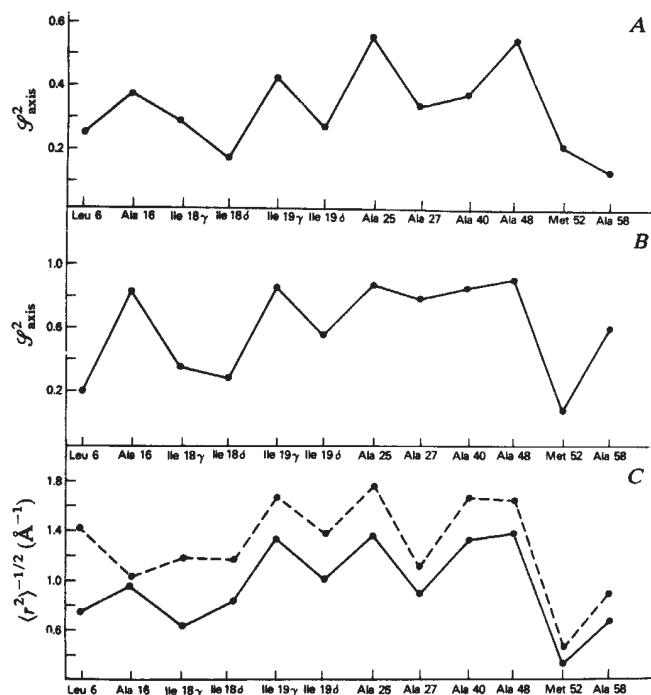


Fig. 1 Comparison of experimental order parameters (A) with those calculated from a molecular dynamics simulation (B). C shows the reciprocals of the theoretical r.m.s. positional fluctuations for the methyl carbons (solid line) and the adjacent heavy atoms (broken line). The experimental order parameters for the δ -carbons of Ile 18 and Ile 19 are based on our assignment of the resonances (see text).

case, independent of the nature of the motion of the symmetry axis, one has⁷

$$\mathcal{S}^2 = \mathcal{S}_{axis}^2 \left(\frac{1}{2} (3 \cos^2 \beta - 1) \right)^2 \quad (2)$$

where β is the angle between the $^{13}\text{C}-\text{H}$ bond and the symmetry axis and $\mathcal{S}_{axis}$ is the generalized order parameter of the bond joining a methyl carbon and its nearest-neighbour heavy atom. $\mathcal{S}_{axis}^2$ can be calculated from a trajectory by replacing the average in equation (1) by a time average.

Figure 1A shows values of $\mathcal{S}_{axis}^2$ obtained from the values of $\mathcal{S}^2$ extracted⁷ from the experimental relaxation data⁹ using equation (2) with the value of β (69.8°) determined from a neutron diffraction study¹¹ of alanine. The experimental relaxation parameters for Ala 16 and Ala 40 are average values because these resonances were not resolved (we used the same order parameters for these two residues). The δ -carbon resonances of Ile 18 and Ile 19 could be identified (resonances a and b) but were not assigned⁹. In constructing Fig. 1A we assigned resonance a to the Ile 18 δ -carbon so that the relative ordering of $\mathcal{S}_{axis}^2$ of the δ -carbons of Ile 18 and Ile 19 agrees with the simulation (Fig. 1B). Figure 1B shows the values of $\mathcal{S}_{axis}^2$ calculated from a 96-ps trajectory of PTI⁸. With the exceptions of Met 52 and Leu 6, the theoretical order parameters are larger than the experimental ones, indicating that there is usually less motional averaging in the 96-ps simulation than detected in the experiment. Such behaviour will occur when the length of the trajectory is too short for a bond to assume all accessible orientations. If there are activation barriers between various conformations, the time required for a single computer simulation to develop an equilibrium distribution of bond orientations will probably be longer than 100 ps (that is, the trajectory can be trapped in a local minimum). The very small order parameter (0.069) for the S-C β bond of Met 52 calculated from the trajectory seems to be due to the neglect of solvent effects in the simulation. Recently, it has been shown⁵ that the inclusion of solvent leads to a dramatic reduction of the average of r.m.s. displacements of Met 52. Based on the experimental order parameters, we conclude that the motion of the C α -C β bond

of Ala 58 is the least restricted of all the residues studied. According to the theoretical order parameters, however, Ala 58 is the most mobile only among the alanines. It is possible that the experimental order parameter of Ala 58 is inaccurate as it is based on only two measured relaxation parameters whereas the other order parameters, with the exception of those of Ala 16 and 40, are based on three. Although differences do exist, it can be seen from Fig. 1A, B that the relative flexibility of the residues is well described in the simulation. For example, the relative mobilities of all the alanines ($58 > 40 \approx 27 \approx 16 > 25 \approx 48$) is predicted correctly.

Using the model-free approach, an order parameter of 0.87 was extracted from the relaxation data for the α -carbon envelope of PTI. This order parameter, determined by the reorientation of the C α -H vectors, is a measure of the average local flexibility of the backbone. Since to a first approximation the C α -C β bonds are expected to move like the C α -H vectors, we evaluated the order parameters of the C α -C β axis for all protonated α -carbons in PTI. The average was 0.84, very close to the experimental value. Note, however, that the experiment indicates that the α -carbons of alanines move more than the average of all the α -carbons (that is, $\mathcal{S}_{axis}^2 = 0.39$ which is less than 0.87) whereas the molecular dynamics predicts that the motion of the alanines is close to the average (that is, $\mathcal{S}_{axis}^2 = 0.81$ as opposed to 0.84).

Finally, it is of interest to compare the pattern of the values of the order parameter to the trends of isotropic r.m.s. displacements from the average atomic positions for a series of residues. As $\mathcal{S}_{axis}^2$ depends on the concerted motions of two nuclei, in Fig. 1C we show the reciprocals of the r.m.s. displacements of both the methyl carbons and their nearest neighbours calculated from the 96-ps trajectory. We have plotted the reciprocals to facilitate a visual comparison with the order parameters. In general, there need not be a simple connection between the ordering of $\mathcal{S}^2$ and the r.m.s. fluctuations as these quantities reflect motional flexibility in different ways. For example, although the calculated order parameters of Ala 16 and 40 are almost the same, the r.m.s. fluctuations of Ala 16 are greater than those of Ala 40. Nevertheless, by comparing Fig. 1B and C it can be seen that there exist many similarities between the pictures of the relative mobility of residues in a protein derived from order parameters and those derived from r.m.s. fluctuations (for example, Ile 18 is more mobile than Ile 19, and Ala 27 is more mobile than Ala 25 by both criteria).

We have described a novel means of testing molecular dynamics simulations where properties related to motions about individual bonds are compared with their experimental counterparts. To our knowledge, this is the first time that the results of a simulation have been related to observable properties at this level of detail. As more experimental data become available on a variety of protein systems, our approach should prove useful in assessing the range of validity of molecular dynamics simulations.

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1. Frauenfelder, H., Petsko, G. & Tsernoglou, A. *Nature* **280**, 558-563 (1979).
2. Artymiuk, P. J. *et al.* *Nature* **280**, 563-568 (1979).
3. Northrup, S. H., Pear, M. R., Morgan, J. D., McCammon, J. A. & Karplus, M. *J. molec. Biol.* **153**, 1087-1109 (1981).
4. Northrup, S. H., Pear, M. R., McCammon, J. A., Karplus, M. & Takano, T. *Nature* **287**, 659-660 (1980).
5. Van Gunsteren, W. F. & Karplus, M. *Nature* **293**, 677-678 (1981).
6. Levitt, M. *Nature* **294**, 379-380 (1981).
7. Lipari, G. & Szabo, A. *J. Am. chem. Soc.* **104**, 4546-4570 (1982).
8. Karplus, M. & McCammon, J. A. *Nature* **277**, 578 (1979).
9. Richarz, R., Nagayama, K. & Wüthrich, K. *Biochemistry* **19**, 5189-5196 (1980).
10. Levy, R. M., Karplus, M. & McCammon, J. A. *J. Am. chem. Soc.* **103**, 994-996 (1981).
11. Lehman, M. S., Koetzle, T. F. & Hamilton, W. C. *J. Am. chem. Soc.* **94**, 2657-2660 (1972).

MATTERS ARISING

Simulating silicate structures and structural chemistry of pyroxenoids

CATLOW *ET AL.*¹ have recently described a way of calculating lattice energies of different silicate structures in an attempt to predict which single-chain silicate structure would be the most stable for a particular metal cation or for a particular pair of cations. The stabilization energy (the difference of lattice energies calculated) of one structure with respect to another is often a very small fraction ($\sim 0.1\%$) of the total lattice energy (calculated per Si atom) and therefore a considerable degree of confidence in the values calculated is required.

The essentially ionic model presented has an empirical correction for 'short-range' interaction—presumably for orbital overlap or covalency effects. This correction does not appear to take account of the fact that in some cation environments not all oxygen ligands are equivalent; some oxygens are so-called bridging oxygens between silica tetrahedra and some are directly bonded to one Si atom only. Whether the neglect of this variation in short-range interaction is significant enough to cause appreciable error is not clear.

Nevertheless, there are many further tests Catlow *et al.* could make to clarify the quantitative validity of their model. For example, it is misleading to conclude that the most energetically favoured pyroxene or pyroxenoid structure for Mg^{2+} cations is the diopside structure when it is known that a pure Mg pyroxene has an ortho- or clino-enstatite structure and not a diopside structure. A lattice energy calculation for Mg^{2+} in an ortho- or clino-enstatite structure should therefore show that the latter structures are more stable than the diopside one. (The same argument applies to Fe^{2+} .)

However, the most intriguing point is whether such lattice energy calculations are refined enough to predict cation ordering correctly in such silicates. There is now enough experimental evidence of cation ordering (or the lack of it) in olivines and pyroxenes to provide a testing ground for such calculations. Traditionally explanations of cation ordering have been on the basis of ionic size or, in the case of transition metals, in terms of crystal field stabilization effects. Such explanations are sometimes the result of hindsight and are often less than convincing; sometimes they fail altogether. Clearly therefore it would be very useful to be able to carry out an energy calculation from first principles. Such calculations ought then to be able to predict for example that Fe^{2+} is not ordered in the

olivine structure but that Mn^{2+} has a strong preference for the M_2 site—putting Mn^{2+} in the M_2 site rather than the M_1 site should lead to greater stability whereas no appreciable stabilization should occur for Fe^{2+} .

Catlow *et al.* show that the effect of having mixed cations often has a stabilization effect on a particular structure. However, the maximum stabilization will, of course, only be achieved if the correct distribution of cations among the possible sites is chosen. Calculations with different cation distributions should therefore predict the correct one.

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1. Catlow, C. R. A., Thomas, J. M., Parker, S. C. & Jefferson, D. A. *Nature* **295**, 658–662 (1982).

CATLOW AND PARKER REPLY—The comments of Walker on our simulation study of pyroxenoids raise two issues. First, what is the 'accuracy' of the calculated lattice energies; second, what further tests of our method are possible. Regarding the question of accuracy we stress that the methods used by our calculations are exact; there are no errors (above the level of machine accuracy) attributable to our lattice summation techniques. Any 'inaccuracy' arises from inadequacies in our interatomic potentials. Here we note that our potentials perform well in predicting structures of the type of mineral discussed in our article, which supports their reliability in a study of structure discrimination. Moreover, we note that the differences between the energies of different structures are appreciable (that is ~ 1 eV). The fact that these are a small proportion of the total energy does not mean that they are insignificant; indeed the total energy contains large terms which are relatively insensitive to the structure. The position here is analogous to that in quantum mechanical studies of the energies of small molecules, where binding energies, although a small proportion of the total energy may, nevertheless, be reliably calculated. The point raised by Walker concerning the possibility of different potential parameters for bridging and non-bridging oxygen atoms is valid. However, the success of our calculations in accurately predicting structures suggests that this is not a major effect, although refinements of our potentials should clearly take this effect into account.

As further tests of our calculations Walker suggests first that we examine the

enstatite structure of MgSiO_3 which he correctly remarks is the most stable structure of this phase. Such calculations are indeed in progress; and our results find that the enstatite structure is more stable than the diopside structure for MgSiO_3 and FeSiO_3 ; further details of these calculations will be reported shortly. Walker also suggests that we use our techniques to tackle problems relating to cation ordering. It was indeed with the longer-term view of investigating such problems, that we originally developed our simulation methods. The success of our work encourages the extension to the problems discussed by Walker, although we emphasize that the computational demands of such work are considerable.

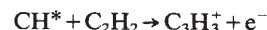
Finally, we should point out that the calculations we reported had the main aim of investigating whether energy minimization techniques could usefully be applied to silicate structural chemistry. We believe that our work established the viability of the technique in this field. Subsequent studies will aim at refining our potential models and hence the accuracy of our predictions.

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S. C. PARKER

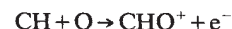
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Chemi-ionization in acetylene flames

EVIDENCE has been presented by Hayhurst and Jones¹ in favour of the preponderance of the reaction



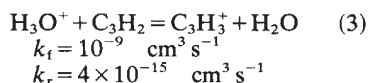
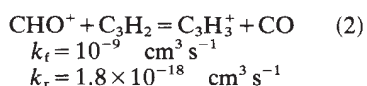
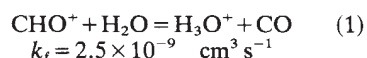
over the reaction



as the primary ionization process in rich acetylene/oxygen flames. This evidence is: (1) low relative concentration of CHO^+ , particularly in the reaction zone; (2) double-peaked profile of C_3H_3^+ in rich $\text{C}_2\text{H}_2/\text{O}_2$ flames; (3) double-peaked profile of total ion current in rich $\text{C}_2\text{H}_2/\text{O}_2$ flames.

All these features may, however, be explained by a mechanism based on CHO^+ alone. It has been realized since the earliest work on mass spectroscopy of flame ions² that rapid proton transfer reactions will deplete CHO^+ in flames so that its concentration relative to prominent ions such as H_3O^+ (from protonation

of water) will be low. Computer simulations of the kinetics of a simple proton transfer scheme have shown that CHO^+ , although the primary ion, will be removed by reaction with H_2O in 10^{-6} – 10^{-5} s (ref. 3). The double-peaked feature in the profile of C_3H_3^+ is also produced by computer simulation of the scheme:



in which the concentration of C_3H_2 , of the order of 10^{-6} mol fraction, is made to follow a double-peaked profile. Reaction (2) was first suggested by Hayhurst and Kittleson⁴. C_3H_2 is an entity about which little is known beyond theoretical calculations of heat of formation, which indicate that it may have a high proton affinity^{5,6}. If it occurs in the reaction zones of acetylene flames in association with CH, it may well also recur in the downstream feather described by Hayhurst and Jones, in association with CH and polyacetylene molecules. Furthermore, there are several ion/molecule reactions involving acetylene and other hydrocarbons which produce C_3H_3^+ (ref. 7).

Finally, the double-peaked profile for the total ion current could be due to a fresh downstream production of CHO^+ (from CH generated in the 'feather') which would then rapidly generate C_3H_3^+ by the mechanism described above.

These points demonstrate the difficulty of finding proof of one mechanism of flame ionization as opposed to another, and the need fully to recognize alternatives in the interpretation of experimental results.

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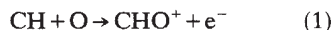
- Hayhurst, A. N. & Jones, H. R. N. *Nature* **296**, 61–63 (1982).
- Green, J. A. & Sugden, T. M. *9th int. Symp. Combustion*, 607 (Combustion Institute, Pittsburgh, 1963).
- McAllister, T. *Proc. 2nd. Aust. Thermodynamics Conf.*, 457–465 (Royal Australian Chemical Institute, Melbourne, 1981).
- Hayhurst, A. N. & Kittleson, D. B. *Combust. Flame* **31**, 37–51 (1978).
- Hehre, W. J. *et al. J. Am. chem. Soc.* **98**, 4378 (1976).
- McAllister, T. & Nicholson, A. J. C. *JCS Faraday Trans. 1*, **77**, 821–825 (1981).
- Kim, J. K., Anicich, V. G. & Huntress, W. T. *J. phys. Chem.* **81**, 1798–1805 (1977).

HAYHURST AND JONES REPLY—We accept "the need fully to recognize alternatives in the interpretation of

experimental results" and in this spirit suggested¹ that



produces ions in addition to the well-established process:



in very fuel-rich oxy-acetylene flames. Our proposal is based primarily on one piece of evidence, that the profile of total positive ion concentration along such a flame's axis displays two maxima. This indicates that there are two spatially separated regions of ion production. McAllister's explanation of this observation is based on both C_3H_2 and ground state CH having double-peaked profiles. The available evidence^{2,3} indicates that the concentrations of C_xH_y intermediates in these flames show only one maximum. In any event, McAllister's scheme involving C_3H_2 is not the only route^{4,5} from CHO^+ to C_3H_3^+ and does not address itself to the generation of ions from neutrals.

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 500 words and the briefest of replies, to the effect that a point is taken, should be considered.

Little quantitative information is available on neutral species in these fuel-rich flames. However, it is known⁶ that the concentration of oxygen atoms is low in the reaction zone and rises to a maximum just before the end of the feather, that is, close to the second maximum in the rate of production of ions, which we attribute¹ to reaction (1). It is likely³ that ground state CH displays one maximum in its concentration somewhere in the feather, but its exact location is not known. Electronically excited CH is possibly unusual⁶ in displaying two concentration maxima—one where the rate of ion production first maximizes and another farther downstream in the feather. Such complications no doubt derive from CH^* being produced³ by $\text{C}_2 + \text{OH} \rightarrow \text{CH}^* + \text{CO}$. The concentration of C_2H_2 declines⁶ steadily through the feather. It is thus clear that the product $[\text{CH}^*][\text{C}_2\text{H}_2]$ has a maximum in the reaction zone, whereas $[\text{CH}][\text{O}]$

has one farther downstream. This is in line with our suggestion¹ that the first ions are produced by reaction (2), but subsequent ones are generated by reaction (1).

The area of any disagreement thus centres on the process responsible for the original ions in the reaction zone of one of these fuel-rich flames of C_2H_2 . Notably, only one oxygenated ion ($\text{C}_2\text{H}_3\text{O}^+$, probably protonated ketene) is detected in the reaction zone. The main positive ion is C_3H_3^+ and in fact all the other ions have the formula C_xH_y^+ . The apparent absence of oxygenated ions, such as H_3O^+ and CHO^+ , suggests that the reactions proceeding in the reaction zone and early part of the feather are largely the pyrolysis of hydrocarbon molecules and radicals, rather than their oxidation, which occurs further downstream⁷. This agrees with the observation⁶ that oxygen atoms have low concentrations in the reaction zone.

The ratio of the rates of reactions (1) and (2) is $k_1[\text{CH}][\text{O}]/k_2[\text{CH}^*][\text{C}_2\text{H}_2]$. The value of k_1/k_2 can be estimated to be around unity. $[\text{CH}^*]$ is much higher than expected on thermodynamic grounds³ and can be expected to have a mol fraction in these reaction zones of $\sim 10^{-8}$. Mol fractions of 0.1 and 10^{-4} can be estimated for C_2H_2 and CH, respectively. This means that for reaction (1) to dominate reaction (2) in the first production of ions, oxygen atoms must have a mol fraction exceeding 10^{-5} , which is most unlikely⁸. Alternatively, the initial rate of production of ions is measured from our¹ Fig. 1 to be around 3×10^{14} ions $\text{ml}^{-1} \text{ s}^{-1}$. If only reaction (2) operates, then the above $[\text{CH}^*]$ and $[\text{C}_2\text{H}_2]$ indicate $k_2 \sim 3 \times 10^{-13}$ ml per ion per s, which is entirely reasonable. Note that k_1 has been measured⁹ to be 2.8×10^{-13} ml per ion per s at the similar temperature of 2,200 K.

Of course, the above discussion in favour of reaction (2) does not prove that it operates. It does indicate, however, that reaction (2) must be taken as a serious possibility in fuel-rich acetylene flames, where conditions are most favourable for it.

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- Hayhurst, A. N. & Jones, H. R. N. *Nature* **296**, 61 (1982).
- Bonne, U., Homann, A. H. & Wagner, H. G. *10th int. Symp. Combustion*, 503 (Combustion Institute, Pittsburgh, 1965).
- Bulewicz, E. M., Padley, P. J. & Smith, R. E. *Proc. R. Soc. A* **315**, 129 (1970).
- Green, J. A. & Sugden, T. M. *9th int. Symp. Combustion*, 607 (Combustion Institute, Pittsburgh, 1963).
- Goodings, J. M., Bohme, D. K. & Sugden, T. M. *16th int. Symp. Combustion*, 891 (1976).
- Ogden, J. E. thesis, Univ. Sheffield (1979).
- Tse, T. S., Michaud, P. & Delfau, J. L. *Nature* **272**, 153 (1978).
- Gaydon, A. G. *The Spectroscopy of Flames* 2nd edn, 264 (Chapman & Hall, London, 1974).
- Peeters, J. & Vinckier, C. *15th int. Symp. Combustion*, 969 (1974).

ANNOUNCEMENTS

Appointments

His Royal Highness The Prince of Wales has been elected to Honorary Fellowship of The Institution of Electrical Engineers.

Two appointments have been made at Queen's University, Belfast, **Dr John Blakeman** has been elected to the Chair of Mycology and Plant Pathology, and **Mr Anthony Valentine** has been appointed to the Chair of Paediatric and Preventive Dentistry.

Dr Bruce A. Reitz, a Stanford University National Science Foundation will take up the position of Chancellor of the College Park campus of the University of Maryland, USA.

Dr Bruce A. Reitz, a Standord University heart surgeon, will become professor and director of the Division of Cardiac Surgery at The Johns Hopkins School of Medicine, Baltimore, USA and cardiac surgeon in charge at The Johns Hopkins Hospital.

Douglas A. Fenderson, director of continuing medical education at the University of Minnesota, has been appointed director of the National Institute for Handicapped Research, USA.

Action on Smoking and Health (ASH) announced that **Professor Peter Sleight**, Field Marshal Alexander Professor of Cardiovascular Medicine at Oxford University, has been appointed its new Chairman.

Dr P W Allen, Assistant to the Director, Malaysian Rubber Producers' Research Association, has been appointed Secretary of the International Rubber Research and Development Board UK.

Dr W. Rizk has been appointed Chairman of the Board of The British Standards Institutions (BSI). He succeeds Mr Paul Fletcher who has retired after completing a three year period of office.

Professor Yadin Dudai has been named first incumbent of the Michael and Sara Sela chair in Neurobiology, recently established at the Weizmann Institute.

Dr Robert Lickley has been elected into Honorary Fellowship of the Institution of Mechanical Engineers.

Maximilian Buja, professor of pathology at The University of Texas Health Science Center at Dallas USA was named the first A.J. Gill Professor of Pathology at the health science center.

The Open University has appointed **Roger Mills** as director of its London regional office.

Awards

The Sero Prize in Steroid Endocrinology for 1982 was presented to **Mr Aristidis Slavakis**, a Greek student, on the MSc Course in Steroid Endocrinology at Leeds University, UK.

The British Society of Rheology has made its Annual Award for 1982 to **Professor F.M. Leslie** of the University of Strathclyde in recognition of his contributions towards an understanding of the rheology of liquid crystals.

Dr Karl Frank Austen, of Harvard Medical School, USA, and **Dr Bengt Ingemar Samuelsson** of the Karolinska Institute in Stockholm, Sweden have been selected to receive the 1982 Waterford Biomedical Science Award for their work in allergy and inflammation.

The international award in the BP Energy Research Prize competition has been awarded to **Dr R.P. Howson**, department of physics, Loughborough University of Technology, for his project on selective optical coatings on plastic sheets.

Mr David Attenborough has been awarded the 1981 Kalinga Prize for the Popularization of Science jointly with **Mr D. Flanagan**, editor of *Scientific American*. The award was established by UNESCO in 1951 by means of a grant from the Kalinga Foundation Trust.

Professor H.H. Hopkins, Professor of Applied Optics in the University of Reading, has been awarded the 1982 Gold Medal of the International Society for Optical Engineering for his contributions spanning a broad range of theoretical and applied optical disciplines.

Engelhard Corporation of Iselin, New Jersey USA has been presented with the Council of Environmental Quality's first 'Award of the Decade' for environmental innovation and achievement by American industry. Engelhard was also cited by the United Nations for its contributions to environmental protection.

The Science and Engineering Research Council (SERC) has awarded a special grant of £350,700 for the study of transport and kinetic processes in fixed catalytic reactors. This work, to be undertaken by **Prof. C. McGreavy** (University of Leeds) and **Prof. W.J. Thomas** (University of Bath), is expected to lead to improvements in the design of catalytic reactors.

Whatman Reeve Angel Plc, of Maidstone, Kent, UK., who produce Laboratory Filter and chromatographic Media, are the winners of the 1982 Sedgwick Safety Award for the South East Region.

The Paul-Martini-Prize, founded in order to promote the development of scientific methods for evaluation in the clinical-pharmacological and therapeutical field, was shared 1982 by three German Scientists — **Prof. Dr Gustav Georg Belz** of the Institut für Kardiovaskuläre Therapie in Weisbaden, **Dr Wittich Doering** of the II. Medizinische Klinik des Krankenhauses München-Schwabing and **Dr Jürgen Eberhard Scherberich**, Zentrum der Inneren Medizin at the Klinikum der Johann Wolfgang-Goethe-Universität, Frankfurt.

The Wellcome Trust has announced the 10 new awards of Wellcome Trust Senior Lecturerships; **Dr Jenefer M. Blackwell**, Ross Institute of Tropical Hygiene, London School of Hygiene & Tropical Medicine; **Dr S.G. Cull-Candy**, Department of Pharmacology, University College London; **Dr P.M. Dean**, Department of Pharmacology, University of Cambridge; **Dr Jean Golding**, Department of Child Health, University of Bristol; **Dr D.J. Harvey**, Department of Pharmacology, University of Oxford; **Dr R.F. Hess**, The Physiological Laboratory, University of Cambridge; **Dr Hazel C. Jones**, Department of Zoology, University of Hull; **Dr M.C. Sheppard**, Department of Medicine, University of Birmingham; **Dr D.G. Thompson**, Medical Unit, The London Hospital Medical College; **Dr H.V. Wheal**, Department of Neurophysiology, University of Southampton.

The prizewinners of the Feldberg Foundation awards for 1982 are **Professor D. Oesterheld**, of the Max Planck Institute for Biochemistry, Munich Germany and **Dr G. Radda** of the Department of Biochemistry, University of Oxford, UK. The Feldberg Foundation for German-English Scientific Exchange aims to promote Anglo-German friendship in the medical and biological sciences.

John H. Warden of the Water Research Centre, Stevenage, UK has been awarded the major share of this year's \$10,000 Chemviron Award for environmental improvement. Other winners are two professors from the University of Hanover in Germany, **Dr H. Ruffer** and **Mr T. Slokman**.

Pfizer Central Research have announced that they will make up to six new awards annually of £1,500 each to support young scientists who have carried out meritorious research at British Universities. The work should have potential application in the search for new human or animal health drugs. Two awards will be made available for research in Organic Chemistry, two in Biology, one in Pharmaceutical Sciences and one in Veterinary Research. Further details from: Miss M. Kitney, Pfizer Ltd., Sandwich, Kent. CT13 9NJ, UK.

Entries are invited for a competition to design and construct a voice-controlled robot. The British Computer Society is running a Voice-Controlled Robot Competition is aimed at technicians following TEC HC/HD courses in colleges and polytechnics. The competition is being organized in two stages. Stage one involves production of plans by the end of December 1982. Six designs will then be selected for construction in the second stage, which will conclude in July 1983.

BP PLC, the largest British oil company, has provided the Royal Society, UK with the sum of £30,000 for expenditure over the next twelve months on awards to enable Chinese and British scientists to work side by side, either in joint research projects, or for visits in either direction associated with scientific discussions or seminars, contributing to Sino-British scientific interchange.

Applications are invited for the Rolex Awards for Enterprise 1984. Projects must display a true spirit of enterprise, and fall into the categories of Applied Sciences and Invention, Exploration and Discovery or The Environment. There are five prizes, each of 50,000 Swiss Francs and a gold Rolex chronometer. Application forms from: The Secretariat, The Rolex Awards for Enterprise, PO Box 178, 1211 Geneva 26, Switzerland, before 31 March 1983.

Meetings

3-24 January, **Training Course on Molecular Aspects of Developmental Biology**, Puerto Rico (B.C. Candelas, Dept of Biology, University of Puerto Rico, Rio Piedras, Puerto Rico 00931, USA).

17-20 January, **The Biology and Biochemistry of Cellular Interactions**, Siena (Dr C. Ceccarini, Centro Ricerche, ISVT Scelvo, Via Fiorentina 1, 53100 Siena, Italy).

17-21 January, **Advances in Gene Technology: Molecular Genetics of Plants and Animals**, Miami (Miami Winter Symposium, PMBA Conference, PO Box 016129, Miami, Florida 33101 USA).

20 January, **Institution of Mining and Metallurgy**, London (The Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR, UK).

28 January, **The Challenger Society**, London (The Challenger Society, Institute of Oceanographic Sciences, Wormley, Godalming, Surrey. GU8 5UB, UK).

22-24 November. The meeting on **Cytopathology of Parasitic Disease** is to be held in Caracas, not London as stated in 7 October issue, (The Ciba Foundation, 41 Portland Place, London W1, UK.)

14-18 February, **Safety Training Programme**, Loughborough (The Centre for Extension Studies (KG), Loughborough University, Loughborough, Leics LE11 3TU, UK).

18-23 February, **Chemtech and ORT '83 Exhibition and Conference**, India (Chemtech Secretariat, 210, Dr D.N. Road, Bombay 400 001, India).

22-23 February, **Conference on Cleaner Technologies**, London (Miss Lesley Claff, Oyez Scientific and Technical Services Ltd., Bath House (3rd Floor), 56 Holborn Viaduct, London EC1A 2EX, UK).

21-22 March, **Actions and Interactions of GABA and Benzodiazepines**, London (Mrs Joan Kruger, Department of Pharmacology, University College, Gower Street, London WC1E 6BT, UK).

26-30 March, **Eighteenth International Symposium on Computer Applications in the Mineral Industries**, London (The Conference Office, Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR, UK).

27-30 March, **First European Symposium on Radiopharmacy and Radiopharmaceuticals**, Elanore, Denmark (Isotope Pharmacy, Frederikssundsvej 378, DK 2700 Copenhagen, Bronsho, Denmark).

28-31 March, **Low Energy Ion Beams**, Loughborough (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

30-31 March, **Universities Federation Animal Welfare/Laboratory Animal Science Association Joint Symposium**, London (Dr H.B. Waynforth, The Animal Unit, Charing Cross Hospital Medical School, St Dunstan's Road, London W6 8RP, UK).

10-17 April, **Environment Impact Assessment Symposium**, Crete (PADC Environmental Impact Assessment and Planning Unit, Department of Geography, University of Aberdeen, Aberdeen, Scotland, UK).

11-13 April, **Annual Chemical Congress**, Lancaster (Dr J.F. Gibson, The Royal Society of Chemistry, Burlington House, London W1V 0BN, UK).

11-15 April, **Symposium on European Rocket and Balloon Programmes and related research**, Interlaken (The Secretariat for the Programme Advisory Committee on the ESRANGE Special Project, European Space Agency, D/OPS-HQ, 8/10 rue Mario Nikis, 75738 Paris Cedex 15, France).

12-15 April, **Conference on Stabilisation and Disinfection of Sewage Sludge**, Manchester. (Mr E. Porter, Water Research Centre, Communications Group, Medmenham Laboratory, PO Box 16, Henley Road, Marlow, Bucks SL7 2HD, UK).

12-15 April, **Electrostatics Conference**, Oxford (The Static Electrification Group, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

13-15 April, **Static Electrification**, Oxford (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1X 1QX, UK).

14-15 April, **British Institute of Radiology's Annual Congress and Scientific Exhibition**, London (British Institute of Radiology, 36 Portland Place, London W1N 3DG, UK).

13-15 April, **International Colloquium on Diatomic Molecules**, Oxford (W.G. Richards, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, UK).

17-19 April, **Symposium on Aquatic Toxicology**, Milwaukee (M.B. Cooper, 1916 Race Street, Philadelphia, Pennsylvania 19103, USA).

18-19 April, **Symposium on the Determination of Radionuclides in Environmental and Biological Materials**, Teddington (Mr B.S. Marshall, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London SE1 9NQ, UK).

18-20 April, **Intramolecular Kinetics**, Warwick (Prof. J.P. Simons, Department of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, UK).

18-22 April, **6th Tandem Conference**, Chester (Mrs S.A. Lowndes, Science and Engineering Research Council, Daresbury Laboratory, Warrington, Cheshire WA4 4AD, UK).

19-21 April, **International Workshop for Atmospheric Spectroscopy**, Oxfordshire (Dr R.W. Saunders (IWAS), Rutherford Appleton Laboratory, Chilton, Didcot, Oxon. OX11 0QX, UK).

19-21 April, **Physical Modelling of Multi-Phase Flow**, Coventry (The Organising Secretary, Physical Modelling of Multi-phase Flow, BHRA Fluid Engineering, Cranfield, Bedford MK43 0AJ, UK).

19-23 April, **Energy '83**, Hamburg (Hamburg Messe und Congress GmbH, Jungiusstrasse 13 Messenhaus, Postfach 3023 60, D-2000 Hamburg 36, Germany).

26-28 April, **Biologic Diagnosis and Treatment of Malignancies**, Geneva (Dr W. Hennesen, Scientific Secretary of IABS, Willadingweg 37, CH-3006 Ben, Switzerland).

23-28 April, **The Science of Biological Specimen Preparation for Microscopy and Microanalysis**, Traverse City (Om Johari, Director, SEM Meetings, PO Box 66507, AMF O'Hare, IL 60666, USA).

24-30 April, **Euchem Conference**, Lucerne (Professor J.E. Baldwin, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, UK).

25 April-1 July, **Understanding Climate Course**, Norwich (Dr T.M.L. Wigley, Climatic Research Unit, University of East Anglia, Norwich, NR4 7TJ, UK).

27-30 April, **3rd Sites Conference on Protein Nucleic Acid Interactions**, Sitges (Prof. J.A. Subirana, Escuela T.S. Ingenieros Industriales, Diagonal, 647, Barcelona (28), Spain).